FINAL REPORT

Rocky Mountain Arsenal

DIOXIN/FURAN TIER I Field Study Results in Wildlife Tissues

June 2001

Prepared by the

Rocky Mountain Arsenal (RMA)
Biological Advisory Subcommittee

Prepared for the

RMA Committee

ROCKY MOUNTAIN ARSENAL

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RESULTS IN WILDLIFE TISSUES
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The information and conclusions presented in this report represent the official technical position of the Biological Advisory Subcommittee (BAS) of the Rocky Mountain Arsenal (RMA). This report constitutes the relevant risk assessment portion of the administrative record for this CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act) operable unit in regards to dioxins.

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The BAS is comprised of designated scientists from five parties that provide technical advice on risk assessment to the RMA Committee, the risk management team, as mandated in the CERCLA Record of Decision (ROD) dated June1996 (wherein the BAS was formerly the Conceptual Remedy Biological Subcommittee). The five parties are the U.S. Army, U.S. Fish and Wildlife Service, Shell Oil Company, U.S. Environmental Protection Agency, and the State of Colorado.

Technical support for the final preparation of this report was provided by various parties including: Gannett Fleming, Foster Wheeler Environmental Corporation, Michigan State University, URS Corporation, and the Midwest Research Institute.

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONCLUDED)

FIGURES

TABLES

TABLES (CONTINUED)

TABLES (CONCLUDED)

APPENDICES

MAPS

ACRONYMS

Ah-R	Aryl-Hydrocarbon Receptor
ANOVA	Analysis of Variance
BAS	Biological Advisory Subcommittee
CDPHE	Colorado Department of Public Health and Environment
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
COC	Contaminant of Concern
EPA	U.S. Environmental Protection Agency
ln	Natural Logarithm
LQCP	Laboratory Quality Control Program
MATC	Maximum Allowable Tissue Concentration
MDL	Method Detection Limit
MQL	Method Quantitation Limit
MRI	Midwest Research Institute
MSU	Michigan State University
NOAEL	No Observed Adverse Effect Level
NPL	National Priorities List
OCP	Organochlorine Pesticide
p	Statistical Significance
PARCC	Precision, Accuracy, Representativeness, Completeness, and Comparability
PAH	Polycyclic Aromatic Hydrocarbon
PCA	Principal Components Analysis
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Dibenzo-P-Dioxins
PCDF	Polychlorinated Dibenzofurans
PE	Performance Evaluation
pg	Picogram (1×10^{-12} g)
pg/g	Picograms per gram
ppt	Part per trillion (equal to 1 pg/g)
QA/QC	Quality Assurance/Quality Control
RMA	Rocky Mountain Arsenal
ROD	Record of Decision
SAP	Sampling and Analysis Plan
SARA	Superfund Amendments and Reauthorization Act
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TCDD-EQ	TCDD equivalents (determined by H4IIE-luc bioassay)
TEF	Toxicity Equivalency Factor
TEQ	Toxic Equivalent, expressed as a relative concentration of TCDD
USFWS	U.S. Fish and Wildlife Service
ww	Wet Weight
α	Alpha
β	Beta
%	Percentage
μg	Microgram
>	Greater Than
<	Less Than

EXECUTIVE SUMMARY

Dioxins and furans were first detected at the Rocky Mountain Arsenal (RMA) by a Colorado Department of Public Health and Environment (CDPHE) study of animal tissues and waste materials collected from the post. The RMA Dioxin and Furan Tier I Field Study (the study in this report) was conducted in response to concern about the possibility of dioxins and furans posing an excess risk to wildlife and possibly to people exposed to soils at the RMA. In an overall phased approach, the Tier I Field Study was designed to determine whether dioxins and furans were contaminants of concern (COCs) at the RMA. A COC is a chemical that has both a source above background, and a potential for release from a contaminated site. As part of follow-on investigations at the RMA to reduce uncertainties in residual risks to wildlife, as mandated by the CERCLA (Comprehensive Environmental Response, Compensation and Liability Act) 1996 Record of Decision (ROD), the Biological Advisory Subcommittee (BAS) designed this study.

This study evaluated whether concentrations of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were greater in wildlife tissues on the RMA than at off-post reference locations. The study also included comparisons of patterns and distributions of types of PCDD/F congeners among on-post and off-post reference samples to assess the potential for an RMA-specific pattern, in the event that statistically significant differences between on-post and off-post groups were not observed in this Tier 1 Screening Study. If PCDD/Fs were found to be greater on the RMA, or if the patterns of PCDD/Fs were different, then further investigation would be considered to better understand the nature, extent, and magnitude of the contamination.

Three indicator species were chosen for this Tier I Screening Study: the American kestrel, the great horned owl, and the common carp. Controlled sample collections of carp and kestrel eggs were carried out, while collection of livers from great horned owls relied on fortuitously (widespread time and uncertain residency) collected samples. These species were selected to reasonably represent terrestrial and aquatic species that would be expected to integrate exposure to PCDD/Fs over discrete areas and prolonged times. Wildlife were collected, rather than soil, to increase the likelihood of finding potential PCDD/F sources at the RMA. Use of wildlife as biomonitors is generally a more efficient method of screening for bioaccumulative contaminants over large spatial areas, such as at the RMA.

Kestrels were selected because egg concentrations had been shown to correlate with gradients of organochlorine pesticide (OCP) concentrations in soil on-post. Great horned owls were selected because of detection of PCDD/Fs in owls analyzed for the prior CDPHE study and because their diet differs from that for kestrels. Carp were selected to represent exposure to aquatic organisms. A total of 46 American kestrel eggs, 26 great horned owl livers, and 18 samples of carp eggs were analyzed for possible elevations of PCDD/Fs from the RMA tissue samples when compared to samples from representative off-post reference areas in the vicinity of the RMA.

Samples were analyzed for a) toxic equivalent concentrations (TEQ), based on relative potencies compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD or TCDD), of 29 PCDD/F congeners that were measured by trace chemical methods, and b) 2,3,7,8-TCDD biologically equivalent concentrations (TCDD-EQ) that were measured by a cell culture bioassay that

integrates the activity of all TCDD-like chemicals. The TEQ instrumental analysis included measurements of 12 TCDD-like polychlorinated biphenyls (PCBs) for purposes of reconciling mass balances of TEQ concentrations with TCDD-EQ responses, since the TCDD-EQ can respond to any chemical with aryl-hydrocarbon receptor (Ah-R) binding activity, including agonists and antagonists.

A Decision Procedure was designed a priori to evaluate the results and for the proper integration of all the results of the different analyses for the three indicator species. Additional samples were analyzed for quality assurance/quality control purposes, and all data were validated to ensure adequate data usability and acceptable compliance with the RMA Data Quality Objectives as specified in the sampling and analysis plan (SAP) for the study.

American Kestrel Results and Conclusions

The major findings of the American kestrel egg study were:

- Concentrations of PCDD/Fs, determined chemically or by the bioassay, were not greater in tissue samples from the RMA than in samples collected from off-post reference areas.
- There were also no significant differences between concentrations in kestrel eggs collected in the core area (Sections 1, 2, 25, 26, 35, and 36 in **Figure 2**) of the RMA and those collected from the peripheral areas of the RMA.
- No unique pattern of PCDD/F congeners that would distinguish on-post samples from off-post reference samples could be identified. However, pattern analysis was complicated by the fact that detection limits for PCDD/F congeners varied substantially among samples, and results and conclusions of the pattern analysis should be interpreted with caution.

The conclusion from the analyses of kestrel eggs was that there is no indication of exposure, beyond background concentrations, to a possible source of PCDD/Fs at the RMA.

Great Horned Owl Results and Conclusions

The major findings of the great horned owl liver study were:

- Concentrations of PCDD/Fs appeared to be substantially elevated in the livers of the four adult great horned owls that were collected on the RMA; however, three of these adult owls were collected in an emaciated (severely thin) condition stemming from a probable diagnoses of dieldrin poisoning or infectious disease. Such a weight loss has been found to cause a translocation of body burdens of PCDD/Fs to the liver, causing artificially higher concentrations to be detected. Therefore, concentrations were adjusted downward. When this was done, the concentrations observed were more similar to those expected had the owls not been emaciated. When the concentrations of PCDD/Fs in owl livers corrected for weight loss were compared, concentrations in adult owl livers from the RMA were slightly greater than those from off the RMA based on borderline (equivocal based on weight of evidence, see **Tables A** and **16**) for statistical differences.
- There were no statistically significant differences between concentrations of TEQs in livers of juvenile owls collected on the RMA and those collected off the RMA.

- The greatest observed concentrations of PCDD/Fs in all age classes (adults, unknown, and juveniles) were found in owls collected in the vicinity of South Plants. However, the relevance of this observation is uncertain due to confounders such as small sample size, deficits in spatial representation, and uncertain residency status for adult owls that can range off-post.
- The results of the bioassay analyses were consistent with the results of PCDD/F TEQs as determined by chemical analyses, in that the owls containing the greatest concentration of TEQs also contained the greatest concentrations of TCDD-EQ.
- The pattern analysis indicated no evidence that a specific PCDD/F congener profile is present in on-post owl samples compared to off-post reference samples. However, the sample size was likely too small to have detected differences in patterns even if they existed.

The results for owls are statistically inconclusive, in general, because of small sample sizes and limitations on the usability of the data that resulted from the fortuitous manner of sample collections, a lack of adequate spatial representativeness of the samples, emaciation of on-post adult owls, and the lack of agreement between results for adults and juveniles. Based on parametric statistical analyses, concentrations of PCDD/Fs appear to be significantly greater in livers of the four adult great horned owls collected on the RMA. However, non-parametric statistical analyses of the same data did not indicate a difference between concentrations of PCDD/Fs. Thus, results are equivocal for owls but suitable for Tier I screening purposes. Furthermore, when one considers exposures from future land uses of the central area of the RMA where greater concentrations were found in owl livers, this exposure pathway is anticipated to be substantially diminished as co-located sources will be minimized or eliminated through remediation.

Carp Results and Conclusions

The major findings of the carp study were:

- The concentrations of PCDD/Fs were very low, near the method detection limit (MDL) of 1 to 2 picograms TEQ/gram parts per trillion wet weight for all samples both on-post and off-post.

- The statistical power of the analyses was less than required for valid comparisons, due largely to the small sample size of off-post fish, but the concentrations of PCDD/Fs measured in the fish from on-post work were as low as background concentrations observed in off-post reference locations, as well as similar to background concentrations measured in national and global surveys (EPA 1992, Buckland et al. 1998).

The conclusion from the analyses of carp eggs was that there was no indication of exposure to a potential source of PCDD/Fs at the RMA. Concentrations of PCDD/Fs in the carp eggs were sufficiently low in both on-post and off-post locations that further analysis is not warranted.

Risk Analysis

Of the three species evaluated, only the adult great horned owls may have been at some level of higher risk from exposures to PCDD/F at the RMA, based on comparisons of concentrations in liver to predetermined maximum allowable tissue concentration (MATC) values. It should be noted that some owls collected from off-post reference locations also had concentrations of PCDD/Fs exceeding the MATC values. It is uncertain whether there were any incremental (in addition to dieldrin related) or excessive risks to adult owl populations from over-exposures to PCDD/Fs at the RMA because adult on-post owls were only slightly more exposed than off-post adult owls.

Conclusions

The BAS concludes from this Tier I Screening Study that there is no evidence to indicate a large bioavailable source of PCDD/Fs on the RMA. There is also insufficient evidence to indicate that PCDD/Fs are definitely COCs at the RMA; however, the data collected from the chemical analysis of livers from great horned owls found in the South Plants area suggests slightly greater adjusted concentrations of PCDD/Fs in liver tissue when compared to owls from most other sampled locations. In addition, two owls from the earlier CDPHE study (EcoLogic 1996) were collected from the same core RMA area and also had relatively great concentrations of PCDD/Fs.

Furthermore, the corresponding dioxin soil study (EPA 2000a) results showed similar patterns of localized elevations of PCDD/Fs in the same core RMA area, as indicated by the wildlife tissues, but not in soils from peripheral locations from the RMA (EPA 2000b). Thus, multiple lines of evidence suggest a localized, low magnitude source of PCDD/Fs in soil and tissue media in the central RMA *core* area of the former South Plants. This area is currently being remediated, and it is the opinion of the BAS that these activities will remove the likely source of these chemicals and should eliminate further exposures along with future risks.

The BAS concludes that the available data and analyses, in consideration of the RMA remediation plans, are sufficient to support the decision that exposure pathways to PCDD/Fs from possible RMA sources will be minimized or eliminated, thus eliminating unacceptable potential risks from these chemicals. The ongoing U.S. Fish and Wildlife Service Biological Monitoring Program could also be used to confirm the effectiveness of the remedy. However, there is a reasonably high degree of certainty that future exposures and risks to wildlife and people at the RMA will be in the low range of local background levels.

1.0 INTRODUCTION

This report presents the evaluation and synthesis for results from different analytical techniques and statistical procedures for the Rocky Mountain Arsenal (RMA) Dioxin/Furan Tier I Field Study. The Tier I Field Study (the study in this report) was conducted in response to concern about the possibility of dioxins and furans posing an excess risk to wildlife and possibly to people exposed to soils at the RMA. In an overall phased approach, the Tier I Field Study was designed to determine whether dioxins and furans were contaminants of concern (COCs) at the RMA. A COC is a chemical that has both a source above background, and a potential for release from a contaminated site. As part of follow-on investigations at the RMA to reduce uncertainties in residual risks to wildlife, as mandated by the CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act) 1996 Record of Decision (ROD), the Biological Advisory Subcommittee (BAS) designed this study.

The Tier I Field Study was conducted as part of a possible two-phased program to resolve the three decisions, posed as questions below, that are outlined in the final (draft prepared prior to sampling) Dioxin/Furan Tier I Field Study Sampling and Analysis Plan (SAP) (BAS 2000).

Question 1: *Are polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) COCs at the RMA?*

Question 2: *What is the incremental risk to biota caused by the presence (if found) of PCDD/Fs at the RMA?*

Question 3: *If determined to be COCs, do PCDD/Fs pose unacceptable ecological risk, as defined by Superfund health-protective criteria (e.g., population sustainability and community integrity), to the RMA populations?*

To begin to address the above three questions, the Tier I Field Study was designed to determine whether PCDD/F concentrations are greater on-post compared to locations off-post. A Decision Procedure was developed to formalize the methods to be used for evaluating the chemical residue and H4IIE-luc bioassay data on PCDD/Fs in samples of wildlife tissues.

The Tier I Field Study program involved the analysis of American kestrel eggs, great horned owl livers, and carp eggs that were collected on the RMA and surrounding off-post reference areas for the main purpose of screening for higher exposures to PCDD/Fs at the RMA areas. The BAS agreed that the primary receptors of interest for biomonitoring of PCDD/F exposure are raptors because of their greater bioaccumulative potential, previously detected concentrations of PCDD/Fs in great horned owl tissues in a study conducted by the State of Colorado (EcoLogic 1996), and because they are resident species of the RMA and surrounding areas. Additionally, great horned owl specimens were also readily available from the U.S. Fish and Wildlife Service (USFWS) fortuitous specimen program, and the kestrels were already being monitored by USFWS for organochlorine pesticide (OCP) accumulation. The same species may or may not be relevant for phase II work.

1.1 History of the RMA

The RMA is a 27-square-mile U.S. Army facility located northeast of Denver, Colorado (illustrated below). The RMA was established in 1942 to manufacture chemical warfare agents and other agent-filled munitions, and to produce incendiary munitions for use in World War II. All manufacturing plants and associated facilities were located in the center of the 17,000-acre post. Production at the center of the RMA had little effect on the wildlife in the surrounding buffer zones. These outlying areas provided undisturbed, formerly agricultural, habitat for many species of wildlife.

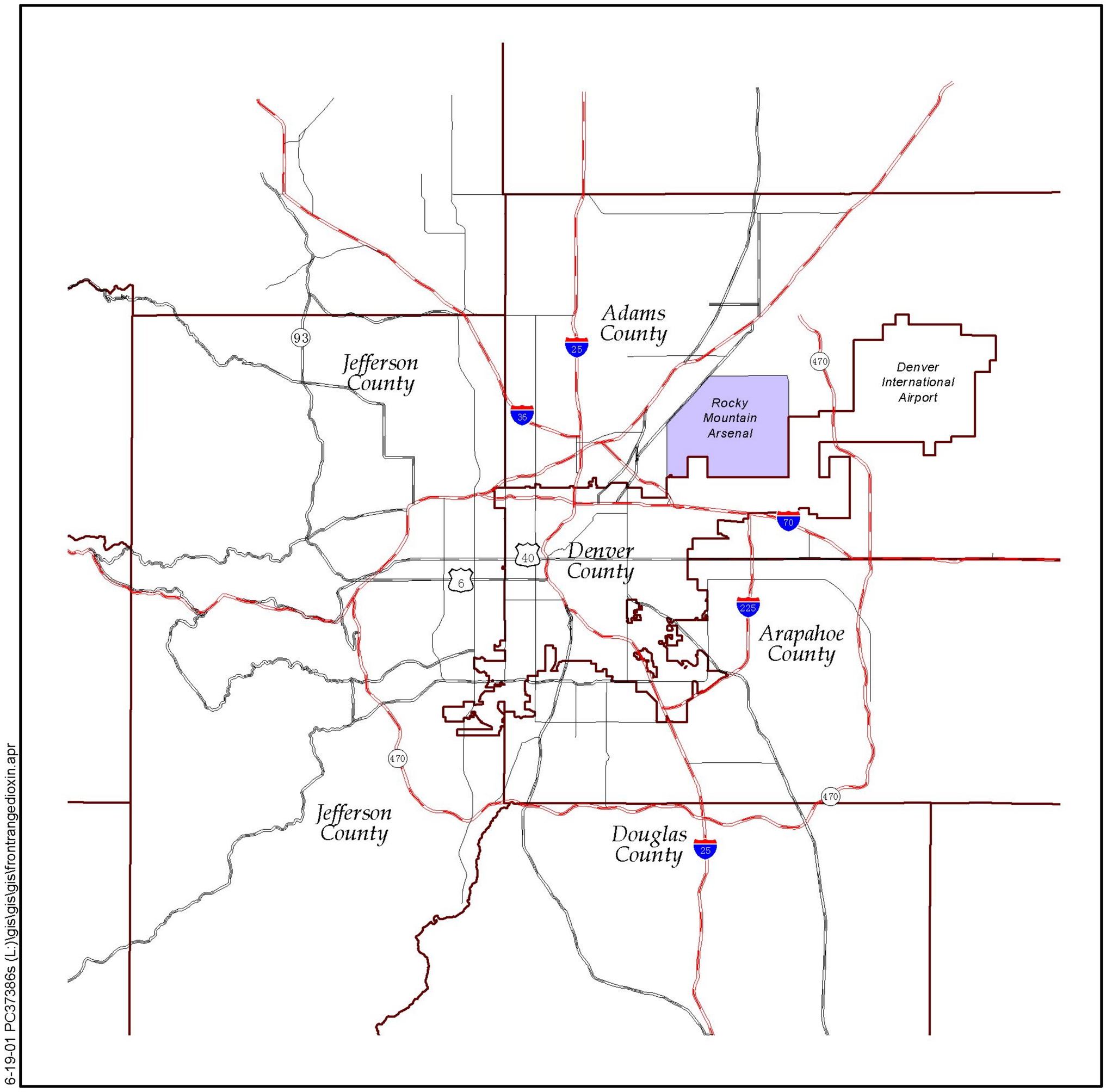
During World War II, mustard gas and chemical munitions were manufactured at the RMA. During the 1950s, Sarin nerve agent was produced. From the 1950s through the 1980s, obsolete and deteriorating ordnance was demilitarized either by neutralizing the contents and burning the remains or by controlled detonation and open burning. Rocket fuel was prepared and stored at the RMA between 1961 and 1982.

Following World War II, portions of the RMA were leased to private industry, primarily for the production of pesticides. Nine companies conducted manufacturing or processing operations in South Plants between 1946 and 1982. The two major leasees of facilities in South Plants were Julius Hyman and Company (Hyman) (1947–1954) and the Shell Chemical Company (Shell) (1954–1982).

Hyman manufactured the chlorinated pesticides aldrin, dieldrin, and chlordane, and also manufactured or brought to the RMA the feedstock chemicals used in manufacturing these products. The feedstock chemicals included hexachloropentadiene, bicycloheptadiene, dichloropentadiene, cyclopentadiene, hydrogen peroxide, acetylene, and chlorine. In 1954, Hyman merged with Shell. Following the merger, Shell leased and constructed additional facilities in South Plants. Shell produced chlorinated hydrocarbon insecticides, organophosphate insecticides, carbamate insecticides, herbicides, and soil fumigants at the facilities in South Plants. No 2,4,5-T or 2,4-D herbicide products, which can contain dioxins and furans, were reportedly produced at the RMA.

Chemical byproducts from these various activities were introduced into the environment at the RMA. Contamination ensued primarily through the burial or surface disposal of solid wastes, discharge of wastewater to unlined or asphalt-lined basins, and leakage of wastewater and industrial effluents through demilitarization activities, routine application of pesticides, and accidental chemical spills, and other releases.

In 1968, the U.S. Army Materiel Command requested recommendations from the National Academy of Science on chemical agent disposal methods. Beginning in 1975, the primary mission of the Army at the RMA was to demilitarize and dispose of obsolete chemical munitions. Shell Chemical continued to lease production areas until 1982, after which all production ceased. In 1980, the mission of the RMA was further refined to direct the disposal of chemical agents and hazardous materials, and decontamination and cleanup of the installation. In 1988, the Secretary of the Army placed the RMA on inactive status and announced that the sole mission of the RMA was cleanup of hazardous contamination. **The illustration on the next page shows the location of the RMA in relation to the Denver Metropolitan area.**



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The RMA was placed on the EPA National Priorities List (NPL) in 1987 and is currently being cleaned up under the authority of CERCLA Act of 1980 and the Superfund Amendments and Reauthorization Act of 1986 (SARA). In 1996, the on-post ROD, which specifies how the RMA will be cleaned up, was signed (FWENC 1996). In October 1992, in conjunction with the future goal of beneficial public use and in recognition of the unique urban wildlife resources and habitat provided by the RMA, President George Bush signed the RMA National Wildlife Refuge Act. This act designates most of the RMA to become a National Wildlife Refuge following U.S. Environmental Protection Agency (EPA) certification that requires remedial actions are appropriately completed to prevent excess site risks.

1.2 Study Background

Based on concerns about the possible presence of PCDD/Fs on the RMA and their availability for exposure to wildlife, the Colorado Department of Public Health and the Environment (CDPHE) sponsored the analysis of Basin F waste and biota samples available from the RMA. The samples were analyzed for trace organic and inorganic compounds, including PCDD/Fs, arsenic, and mercury (EcoLogic 1996). The independent contract laboratory was not required to meet the Data Quality Objectives and quality assurance (QA) procedures mandated for data collected in other RMA studies, such as was required in this current study, and thus the data were not appropriate for confident decision-making purposes. Results of these analyses are summarized in **Appendix** A.

A series of drums containing wastes from the former Basin F were sampled. In three of the four drums analyzed, PCDD/Fs were not measurable above the method detection limit (MDL) of 200 to 300 picograms per gram (pg/g, equivalent to parts per trillion [ppt]). Only three congeners of 17 analyzed were detected in these three waste samples. These were the relatively ubiquitous, higher chlorinated PCDD/F congeners with low toxicity. The fourth waste sample had detection limits approximately 10 times lower than the other three. In this sample, 14 of 17 congeners were detected resulting in an estimated total toxic equivalent (TEQ) concentration of 78 pg/g.

The biota samples were collected from dead animals found on the RMA from 1989 through the end of 1991. The biota samples that were analyzed by the CDPHE study were: three great horned owls, one red-tailed hawk, one 13-lined ground squirrel, three deer mice, and one brown bat. The PCDD/Fs found in Basin F waste and in some of the biota samples helped lead to the current Tier I Field Study. The BAS was directed by the RMA Committee to conduct a more comprehensive investigation of the PCDD/F issues in accordance with the RMA On-Post ROD, Section 6.2.4.3, Continuing Biological Studies (FWENC 1996). The BAS recommended focusing first on potential exposure to wildlife as bioindicators, rather than directly analyzing many expensive soil samples at the RMA.

In pursuing the RMA Committee's directive, the BAS decided on a phased scientific approach. The overall purpose of the PCDD/F study was to determine if concentrations of PCDD/Fs in representative biota samples collected on the RMA were significantly greater than those in comparable samples from off-post reference sites. Besides achieving this purpose, PCDD/Fs were also evaluated for their potential to be a COC, by comparing levels and patterns of PCDD/Fs found in biological tissues collected from the RMA and from off-post reference areas. The BAS agreed to conduct an initial PCDD/F screening study (Tier I Field Study) of wildlife

tissue exposure, the results of which would be used to assess whether further sampling or other studies (subsequent tiers) would be needed to achieve the aforementioned purposes; i.e., refer to the three risk decision questions at the beginning of this introduction section.

A Decision Procedure was designed (**Appendix** B) to provide statistical interpretations of concentrations of the 17 PCDD/Fs and 12 polychlorinated biphenyls (PCBs) with Ah-R agonist activity, and to evaluate criteria for conclusions about the possible outcomes of combinations for various results from the testing of specific hypotheses. This Decision Procedure was used to assess whether defensible risk-based decisions could be made with the Tier I Field Study data, or if not, whether additional analysis of other biota samples and possibly abiotic studies would be needed to support sound remedial decisions at the RMA.

2.0 SAMPLE COLLECTION PROTOCOL

The information for the collection and handling of all species for this Tier I Field Study is discussed in detail in Section 3.1 of the SAP (BAS 2000). The information below is a synopsis of the plan to assist in interpretation of the data.

2.1 Species Sampling Procedures

Three indicator species were chosen for this study: American kestrel, great horned owl, and carp. Controlled sample collections of carp and kestrel eggs were carried out, while collections of the great horned owls relied on more variable fortuitous samples. Forty-six American kestrel eggs, 26 great horned owl livers, and 18 samples of carp eggs were collected from sites on the RMA and from representative off-post reference areas in the vicinity of the RMA.

2.1.1 American Kestrel

The following procedures were used for the 1998 American kestrel egg sampling efforts. These efforts paralleled those instituted under the USFWS Biomonitoring Program, which was initiated prior to the preparation of the SAP. Kestrels have a moderate home range that is roughly associated with spatially stratified nest box placements at the RMA, and sufficient residency time to accumulate dieldrin (an organochlorine, like dioxin) in tissues of eggs (Figure 1). Kestrels were therefore assumed to be a good candidate species for assimilating measurable PCDD/Fs that could possibly be attributable to the RMA at general locations. The USFWS Kestrel Nest Box Monitoring protocol is presented in Appendix D of the SAP. The nest box locations used for the Tier I Field Study on the RMA are provided in **Figure 2**. The off-post collection locations are provided in **Figure 3**.

After kestrel eggs were collected, they were processed at the USFWS RMA laboratory facility. The contents of each egg were removed by cutting the eggshell with a disposable sterile scalpel blade along the equator of the eggshell, and placed in a specially cleaned Eagle Pitcher 2-ounce glass jar to be frozen. Each sample was given a unique sample number that identified species, matrix, and the nest box from which it was collected. All pertinent sample information such as time, date, collector, unique specimen number, species, location, and condition was recorded. Egg samples were analyzed for the presence of Ah-R agonists with congener-specific analyses (TEQ) and the H4IIE-luc bioassay (TCDD-EQ). Spiked quail eggs, which contained known amounts of PCB-126, were submitted blindly and randomly to the laboratories to test for accuracy of the methodologies.

Measurement of PCDD/F Concentrations

Polychlorinated Dibenzo-*para*-Dioxins and polychlorinated Dibenzo-Furans are complex mixtures of as many as 210 individual chemical congeners—75 PCDDs and 135 PCDFs. The relative concentrations of the congeners vary widely among samples. In addition, the relative toxicity of the individual congeners varies from two-fold to more than approximately 100,000-fold. However, only 7 PCDDs and 10 PCDFs are toxic. Thus, it is not possible to determine the toxicity of these mixtures solely by determining the total PCDD/F concentrations as a sum of the congener concentrations. To determine the potential toxic effects of mixtures of PCDD/F concentrations, it is necessary to combine concentrations of PCDD/Fs into a single aggregated measure of "equivalent toxicity."

The BAS chose to use the conventional toxic equivalency factor (TEF) approach to estimate the total toxicity-based exposure to wildlife. The current scientifically accepted measure that provides this estimate is the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity equivalent (TEQ). In this approach, the toxic potency (TEF, WHO [World Health Organization]) of each congener is expressed as a relative concentration when compared to the most potent Ah-R agonist: TCDD. This approach provides an estimate of the total toxicity of all the congeners that act through a single mechanism of action that is initiated by the binding of chemicals to the cellular aryl-hydrocarbon receptor (Ah-R). The TCDD toxicity equivalent is an estimate of the concentration of TCDD that would have the same toxicity as the mixture of total PCDD/Fs in contaminated samples. In addition to PCDD/Fs, there are other related chemicals that contain some TCDD-like activity, such as PCBs and other poly-halogenated chemicals. The BAS also analyzed for 12 PCBs in tissues, even though PCBs were not considered COCs at RMA for purposes of mass-balance comparisons of results by two methods as described below.

In this study, aggregate measures of toxicity were determined in two ways using two different sets of empirical data. These methods are: 1) chemical residue measurement by instrumental analyses of 29 individual congener concentrations, that are multiplied by the TEFs promulgated by the WHO (van den Berg et al. 1998), denoted here as TEQ, and 2) bioassay determination of TCCD equivalent concentrations of all TCDD-like compounds which elicit a biological response that is mediated by binding with the Ah-R of H4IIE-luc cells (rat hepatoma cells with a luciferase indicator) in laboratory cultures, denoted here as TCDD-EQ. Detailed discussions of these methods are provided in the SAP.

2.1.2 Great Horned Owl

A formal protocol for collection of fortuitous specimens was developed by the USFWS in 1993 (USFWS 1994 and BAS 2000). This protocol was agreed to be adequate and acceptable for this Tier 1 screening investigation. When a great horned owl was found dead or moribund, its carcass was placed into a plastic bag and specific procedures were followed. All pertinent information such as time, date, collector, unique specimen number, species, location, and condition was recorded onto a sample tag and a fortuitous specimen form. The completed sample tag was placed inside a second plastic bag, along with the first bag that contained the collected specimen. Specimens were refrigerated if they were shipped for necropsy within 24 hours of the collection time. If shipment could not occur within 24 hours of the collection time, specimens were frozen until shipping was possible. Freezers that stored specimens were locked and located in a room with controlled access. Chain-of-custody procedures were used when submitting specimens to the analytical laboratories.

Both juvenile and adult great horned owls were analyzed in order to account for any differences in PCDD/F concentrations that may be caused by age. Owls were collected from the RMA and off-post reference areas along the Front Range and from northeastern Colorado. The collection locations of fortuitous owl samples at the RMA that were used for this Tier I Field Study are depicted in **Figure 4**. The off-post great horned owl collection locations are depicted in **Figure 5**

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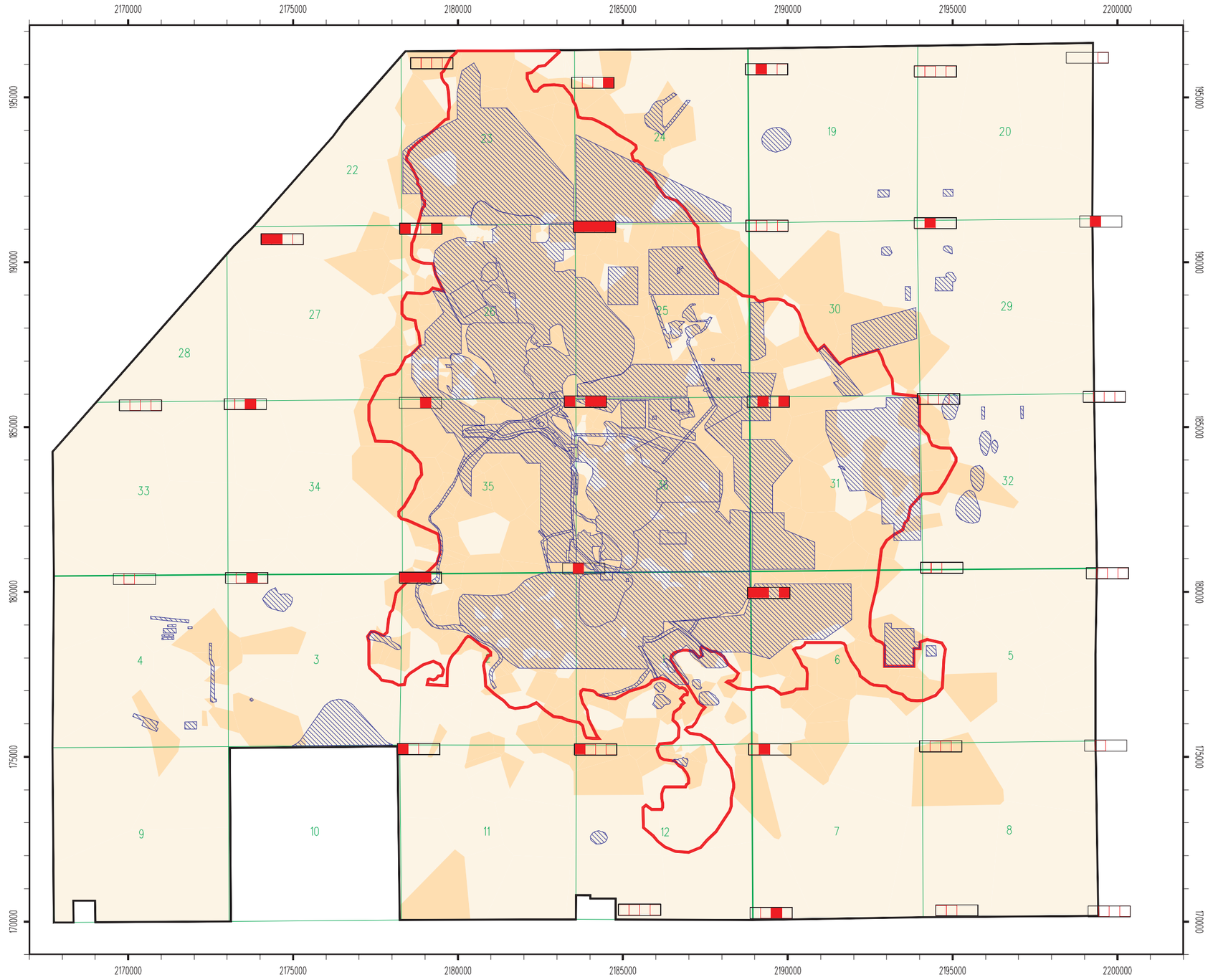


Legend

93 94 95 96 — Each Box Represents a Year, 1993 - 1996

- Dieldrin In Eggs - Value Below .05 ppm
- Dieldrin In Eggs - Value Above .05 ppm
- Dieldrin Surficial Soil Concentrations LE .025
- Dieldrin Surficial Soil Concentrations GT .025 Areas of Potential Residual Risk
- Areas to be remediated
- Area of Contamination (AOC) Boundary

N W E S

Source: U.S. Fish and Wildlife Service

Scale: 1:38400
1 Inch = 3200 Feet

0 1600 3200 Feet

State Plane Coordinate System, CO North Zone
NAD27-NGVD29 Datum

Rocky Mountain Arsenal
Commerce City, Colorado

Dieldrin Concentrations In Surficial Soil

DATE: 10/01/97, AML: /disk05/projects/usfw/kestrels/amls/dld_egg.aml

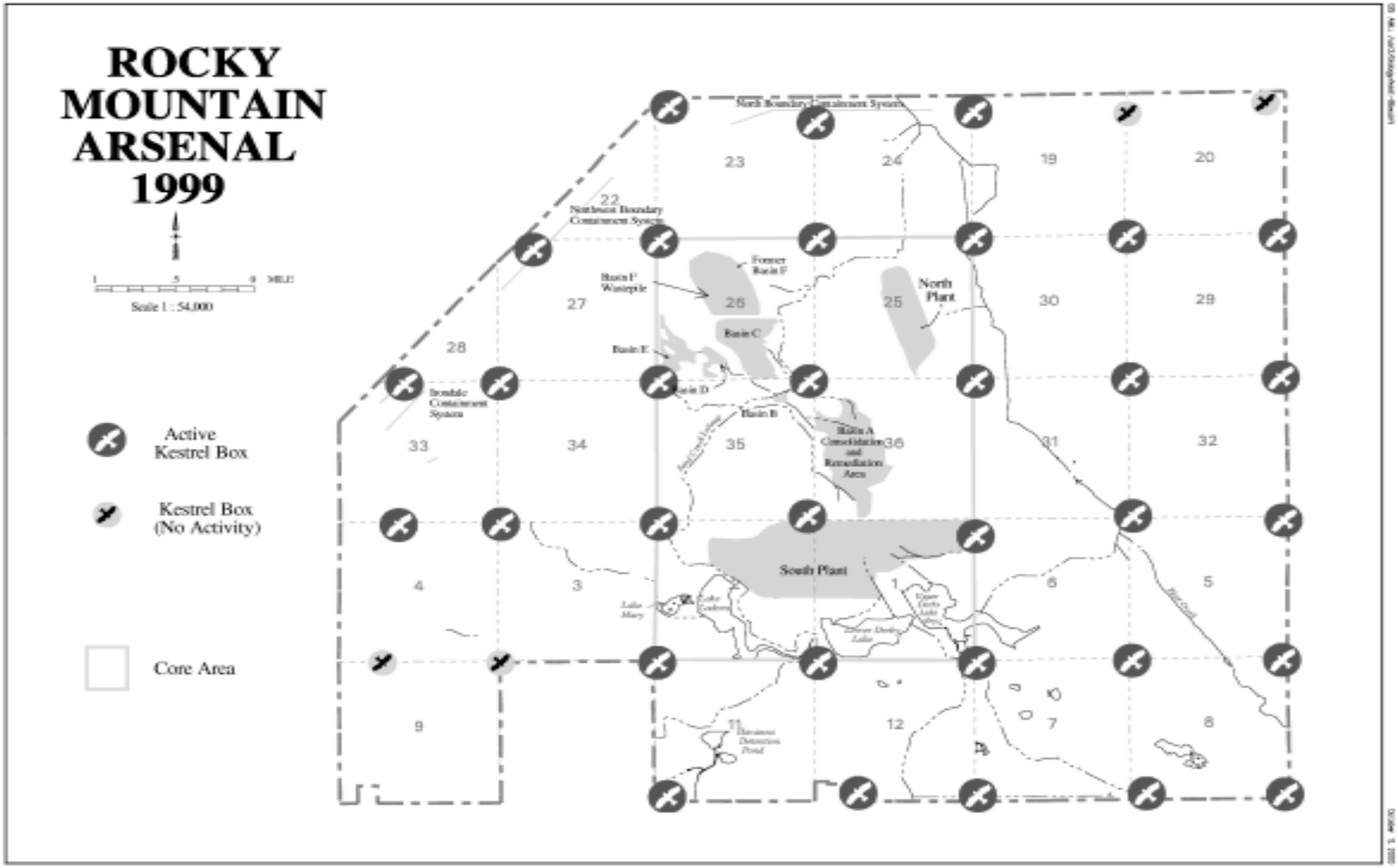


Figure 2 Kestrel Nest Box Locations

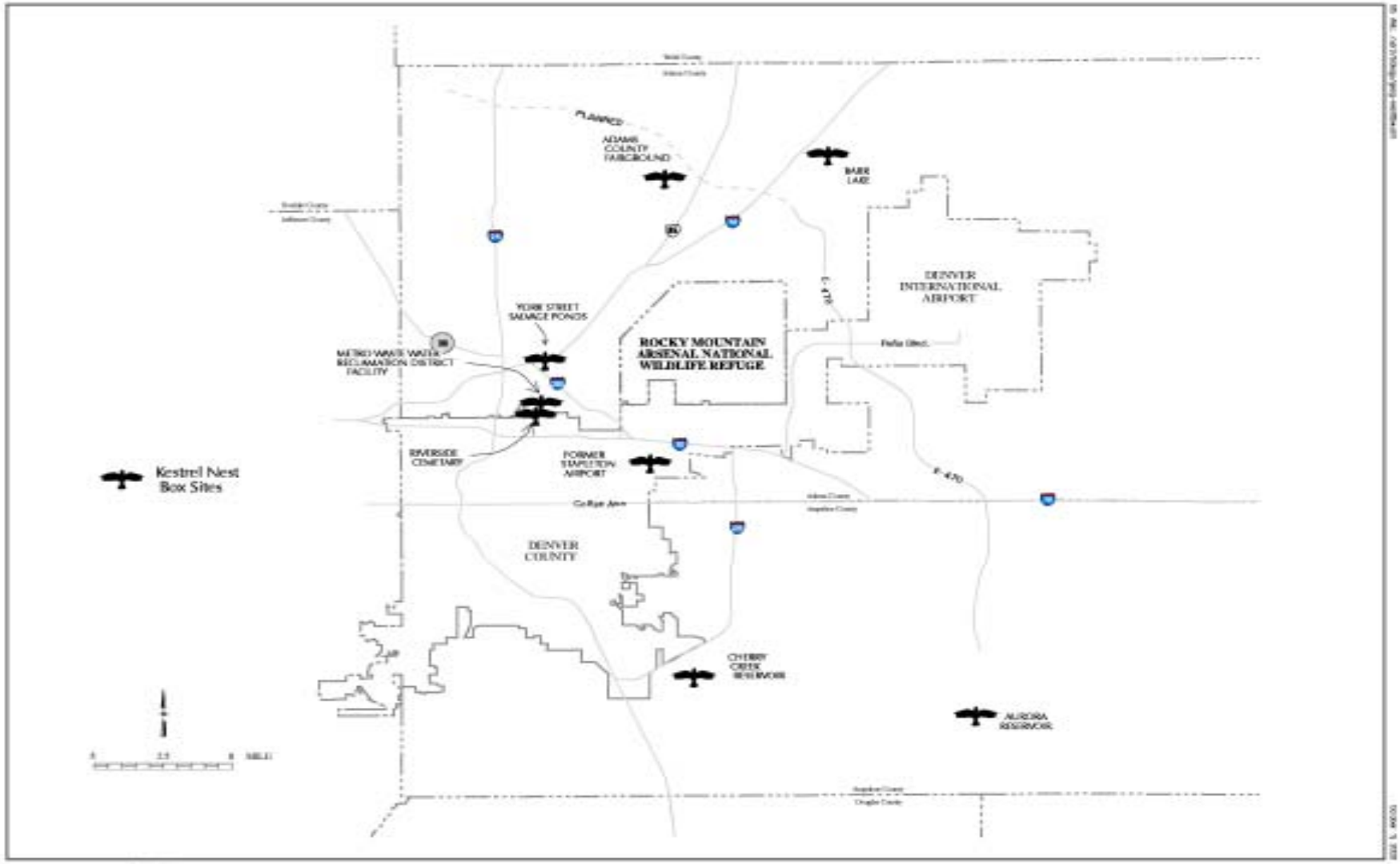


Figure 3 Kestrel Off- Post Nest Box Locations

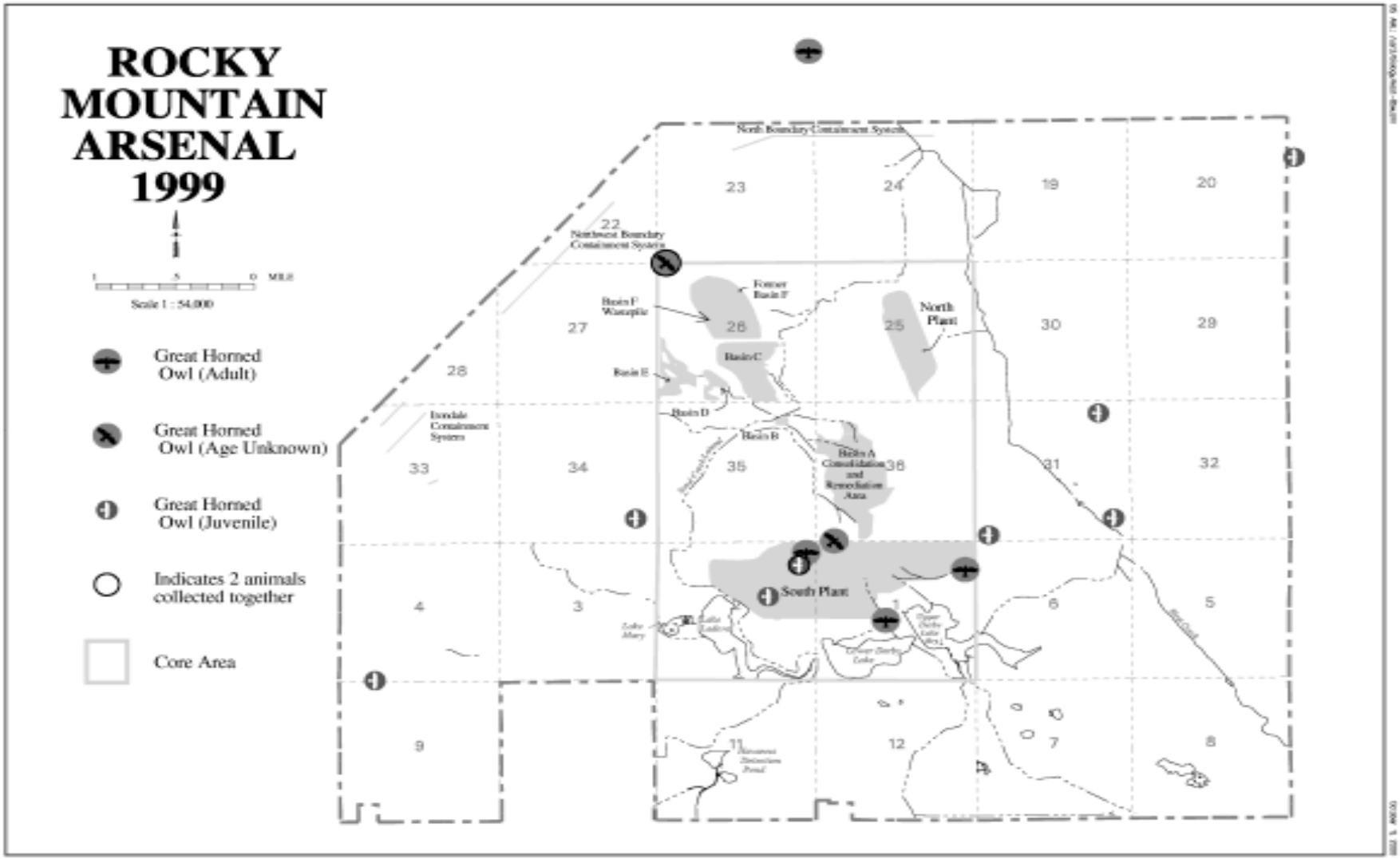


Figure 4 Owl Collection Locations

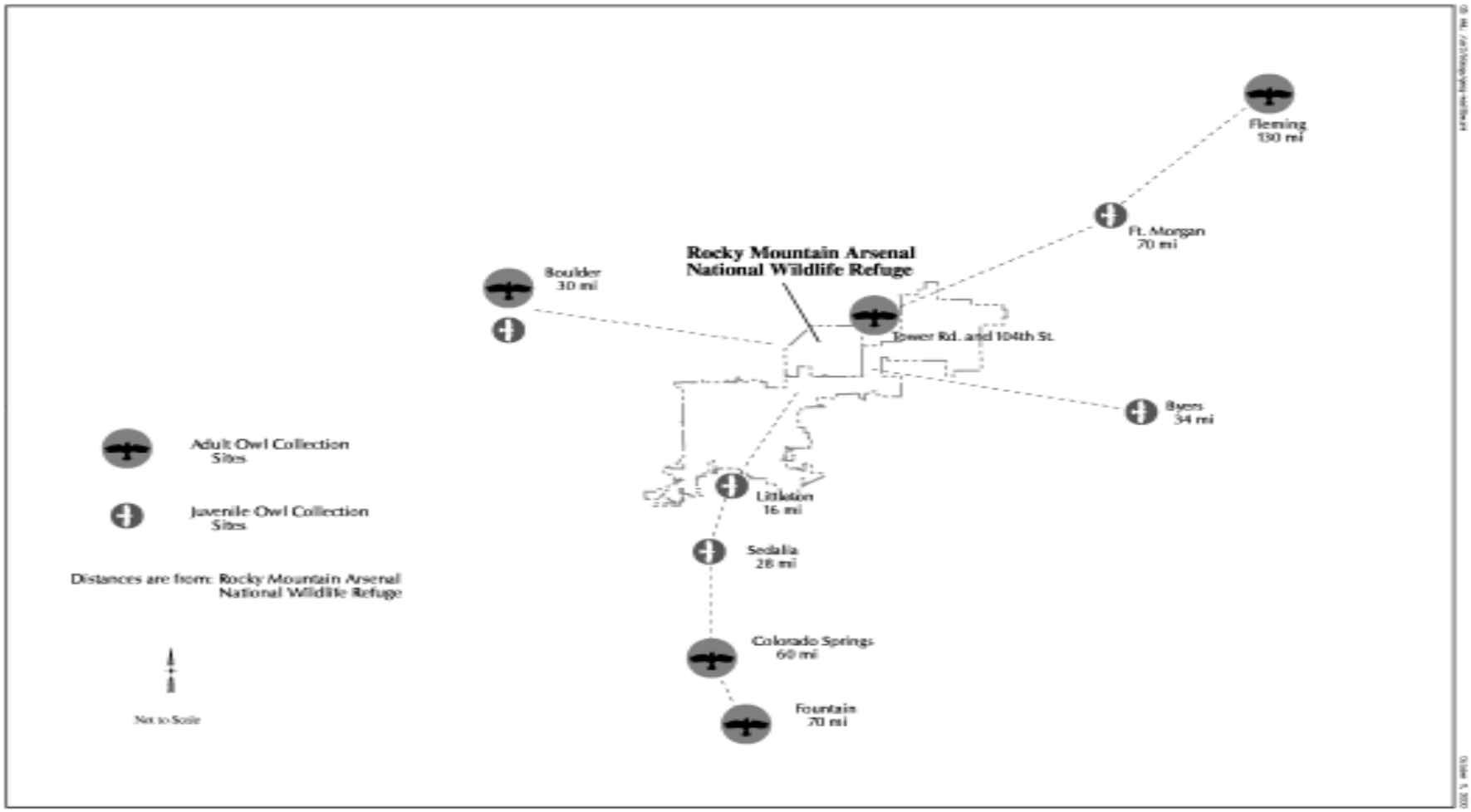


Figure 5 Off- Post Collection Locations for Great Horned Owls

2.1.3 Carp

Carp egg masses were collected from a total of 18 sexually mature female carp in the spring. To control for age and exposure potential, only carp measuring between 18 and 26 inches long were collected. On-post carp were collected from Lower Derby Lake (see **Figure 2** for lake location). Off-post reference carp were collected from Banner Lakes.

Carp were collected by use of gill nets and electro-shocking techniques. Following capture, fish were temporarily stored in a live well until euthanized. Egg masses were collected directly from the fish and placed in specially prepared glass jars.

All pertinent sample information such as time, date, collector, unique specimen number, species, location, and condition (length and body weight) were recorded on a sample tag for each collected sample. Any other tissues that were removed were also recorded on the sample tag.

Carp carcasses (without the eggs) were wrapped in hexane/acetone-rinsed aluminum foil and archived in a controlled setting until all analytical results were evaluated. Eggs were submitted for the H4IIE-luc bioassay (TCDD-EQ) and congener-specific analyses (TEQ).

2.2 Laboratory Standard Operating Procedures

The Standard Operating Procedures (SOP) for the Congener-Specific Preparation and Analysis of PCDDs, PCDFs, and PCBs was prepared by Midwest Research Institute (MRI) and is included in Appendix B of the SAP (BAS 2000).

The full Standard Operating Procedures for the H4IIE-Luc Bioassay, prepared by Michigan State University (MSU), is explained in Appendix A of the SAP (BAS 2000).

3.0 DECISION PROCEDURE

The following is a brief summary of the Decision Procedure used to evaluate the data from this Tier I Field Study. A more in-depth synopsis of the Decision Procedure is included as **Appendix** B of this report. The complete Decision Procedure is Appendix C of the SAP (BAS 2000). The Decision Procedure was used to evaluate the chemical residue analyses of PCDDs and PCDFs (TEQs) and the 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents (TCDD-EQs) determined by the H4IIE-luc bioassay in samples of wildlife tissues, to answer the following question:

> ***Are concentrations of PCDD/Fs in representative biota samples collected on the RMA greater than those in comparable samples from off-post reference sites?***

The first step of the Decision Procedure was to assess the acceptability and usefulness of the data. Quality assurance and quality control (QA/QC) procedures are outlined in the SAP and in the laboratory QC program for each laboratory, based upon performance criteria. The Decision Procedure next specifies how concentrations of PCDD/Fs in biota at the RMA were planned to be statistically compared to concentrations in the same species at off-post reference sites. Three different statistical comparisons of PCDD/F concentrations were made between groups of biota from the RMA and off-post reference areas. The first two comparisons examine differences between concentrations of TEQs and/or TCDD-EQs in biota from the RMA and off-post references, and the third comparison evaluates the pattern of congeners present in each species.

To answer the general question posed above for the Tier I Field Study, greater weight was placed on concentrations of TEQs as calculated from concentrations of PCDD/Fs and TEFs, because these measurements represent more definitive chemical analyses of the target chemicals and are linked to a wider range of environmental fate and effects data. There is also a greater regulatory history and acceptance of TEQs for risk assessment than currently for TCDD-EQs. In addition, while the H4IIE-luc bioassay does not specifically measure dioxins and furans (a disadvantage), it does measures all TCDD-like chemicals (an advantage), including those not targeted by the chemical analyses that act through the Ah-R binding mechanism that can lead to additional TCDD-like toxicity. Thus, the bioassay measures the actual biological activity in a sample, plus it provides a more direct measure of biological relevance of the TCDD-like chemicals present in the sample.

Three approaches were used for statistical comparisons to provide answers for specific Tier I Field Study questions that were scientifically formulated as ***null*** and ***alternative*** hypotheses. The criteria for rejection of the null hypothesis with concomitant acceptance of the alternative hypotheses involve specifying a significance level of probabilities for Type I error (α) to be less than (<) 0.05 (providing confidence [1 - α] as greater than [>] 95%) and probability for Type II error (β) to be < 0.20 (producing power as [1 - β] > 80%). A Type I error is committed when one falsely concludes there is a difference between two groups, when truly there is not a significant difference; i.e., a false positive conclusion is made. Conversely, a Type II error is committed if one falsely concludes there is no difference between two groups, when truly there is a significant difference that was missed for various reasons; i.e., a false negative conclusion is made.

Decision matrices and decision flowcharts were created based on the Decision Procedure used in guiding risk-based decision making, as presented in the Weights-of-Evidence approach described in **Tables A through D** and **Figure 6**.

Table A. Decision matrix for American kestrel eggs and great horned owl livers to support the evaluation of PCDD/Fs as COCs[1] at the RMA

Step V in column 5 below addresses the general question to be answered by the Biological Assessment Subcommittee (BAS) for this Tier I Field Study, stated as: ***Are concentrations[2] of PCDD/Fs in biota samples from the RMA greater than those in the same species collected from the selected off-post reference locations?***

Step I: Data Usability	Step II: TEQ ($H1_o$ or $H2_o$)	Step III: TCDD-EQ ($H3_o$ or $H4_o$)	Step IV: Pattern Analyses ($H5_o$)	Step V: BAS's Answer for Overall Decision[3, 4]	Examples of the BAS's considerations for professional interpretation of the Overall Decision
Evaluated	Reject H_o	Reject H_o	Reject H_o	YES	Probable COC at the RMA.
Evaluated			Accept H_o	YES or Inconclusive	Perform mass-balance[5] with REPs (relative effect potencies).
Evaluated	Reject H_o	Inconclusive	Reject H_o	YES	Probable COC at the RMA.
Evaluated			Accept H_o	YES or Inconclusive	Perform mass-balance[5] with REPs.
Evaluated	Reject H_o	Accept H_o	Reject H_o	YES	Possible[6] COC at the RMA.
Evaluated			Accept H_o	YES or Inconclusive	Perform mass-balance[5] with REPs.
Evaluated	Accept H_o	Reject H_o	NA	Recalculate TEQs including PCBs	After recalculating the TEQs including PCBs, repeat the statistical analysis, and use the sub-matrix below.
Evaluated	Inconclusive	Reject H_o	NA	Recalculate TEQs including PCBs	After recalculating the TEQs including PCBs, repeat the statistical analysis, and use the sub-matrix below.
Evaluated	Inconclusive	Inconclusive	Reject H_o	Inconclusive	May indicate a small local PCDD/F source.
Evaluated			Accept H_o	NO	Uncertain toxicity equivalent factors (TEFs) and trace analysis may be cause for TEQ.
Evaluated	Inconclusive	Accept H_o	Reject H_o	Inconclusive	May indicate a small local PCDD/F source.
Evaluated			Accept H_o	NO	Uncertain TEFs and trace analysis may be cause for TEQ.
Evaluated	Accept H_o	Inconclusive	Reject H_o	Inconclusive	May indicate a small local PCDD/F source.
Evaluated			Accept H_o	NO	Possible non-PCDD/Fs causing slightly higher bioactivity.
Evaluated	Accept H_o	Accept H_o	Reject H_o	Inconclusive	May indicate a small local PCDD/F source.
Evaluated			Accept H_o	NO	Probably not a COC at the RMA.

Table B. Decision Sub-matrix for American kestrel eggs and great horned owl livers to evaluate PCB contributions at the RMA for outcomes when the null hypothesis is rejected for Step III TCDD-EQ but accepted or inconclusive for Step II TEQ

Step V in column 5 addresses the general question for this Tier I Field Study: ***Are concentrations[2] of PCDD/F in biota samples from the RMA greater than those in the same species collected from the selected off-post reference locations?***

Recalculate the TEQ including PCBs for Step II, and then use the following matrix for decision for the overall outcome.

Step I: Data Usability	Step II: TEQ ($H1_o$ or $H2_o$)	Step III: TCDD-EQ ($H3_o$ or $H4_o$)	Step IV: Pattern Analysis ($H5_o$)	Step V: Overall Decision[3,4]	Examples of considerations for interpretation of Overall Decision
Evaluated	Reject H_o	Reject H_o	Reject H_o	Inconclusive	May indicate a small local PCDD/F source; however, PCB congeners account of majority of differences.
Evaluated			Accept H_o	NO	This outcome may indicate that PCB congeners are significantly greater for RMA samples than off-post reference samples. The BAS will consider the implications.
Evaluated	Accept H_o	Reject H_o	Reject H_o	Inconclusive	May indicate a small local PCDD/F source.
Evaluated			Accept H_o	NO	Possible other agonist causing bioactivity.

Notes: (for Tables A and B)

1. COC (contaminant of concern) is an EPA term for a chemical that has both a source and a potential for release from a site, as per EPA Guidance (EPA 1989) that is based on Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and National Contingency Plan (NCP) regulations. The BAS agreed to use a stepwise scientific approach that evaluates the weight and strength of the major "lines of scientific evidence" from tiered biological studies at the RMA, which provide site-specific information to evaluate whether PCDD/Fs may be COCs. Using this stepwise approach to reach the overall decision in Step V above, Step I (not shown) was performed first to ensure the adequacy of data for further valid biostatistical evaluations, and then the BAS considered the anticipated combinations of possible results as shown in Steps II through IV. The possible outcomes in the matrix are sorted in descending order with the strongest evidence for existence of COCs at the top and the strongest evidence for absence of COCs at the bottom, with more weight being given to the results from the TEQ analyses in Step II.
2. Concentration, as used in this context, means "toxic-equivalents" of 2,3,7,8-TCDD that are generated by the 17 PCDD/F congeners with Ah-R agonist activity. It is important to note that only Step II (TEQ) provides results from a direct measure of PCDD/F concentrations, although those measurements can become less certain near the analytical detection limits due to measurement errors and due to uncertainties in TEFs; additionally, Step III (TCDD-EQ) can provide an indirect measure of PCDD/F concentrations, provided that the bioassay results are not overshadowed by other chemicals with Ah-R activity.
3. An "inconclusive" decision indicates that the general question posed cannot be answered as "yes" or "no" with sufficient scientific confidence. An inconclusive outcome will result in further ecotoxicological analysis of the problem by the BAS.

4. The BAS recognizes that bioassay derived TCDD-EQ concentrations might not reflect analytically derived TEQ concentrations because biota extracts may contain substantial amounts of other types of Ah-R agonists or antagonists (e.g., PCBs, polycyclic aromatic hydrocarbons, polychlorinated naphthalenes, etc.). If such other Ah-R agonists or antagonists are present in samples at sufficiently high concentrations, they will likely influence the TCDD-EQ concentrations while not being totally accounted for in the chemical residue analyses. Therefore, while TCDD-EQ results by themselves cannot answer the general question posed in the Tier 1 Field Study, TCDD-EQs can be used in a weight-of-evidence approach to help guide (a) the interpretation of toxicological significance (especially if PCDD/Fs have the predominance of Ah-R activity), and (b) possible future studies at the RMA. The BAS generally recognizes that TCDD-EQs, if not overshadowed by other Ah-R activity, can potentially show differences (similar to TEQs) in PCDD/F concentrations on- and off-post.
5. This overall answer depends on the results of the pattern analyses: (a) if the Principal Components Analysis (PCA) visual patterns and/or cluster analyses and profile analyses of relative concentrations of PCDD/F congeners are the same, but the masses of PCDD/Fs are substantially greater on-post than in off-post samples, then the outcome is "yes," or (b) if the masses are similar in this event, then the outcome is "inconclusive."
6. The suggested interpretation of the outcome for this scenario is downgraded to "possible COC" from "probable COC," because this situation is anticipated to occur from a small difference between groups with relatively low TEQs that may be barely significant ($p < 0.05$); therefore, there would likely be greater uncertainty in this outcome, since the results may be driven by error in trace-level detection limit concentrations coupled with uncertain TEFs.

Table C. Decision Matrix for Combined Results for Terrestrial Species to Support the Evaluation of PCDD/Fs as COCs at the RMA

Column 4 addresses the general question for this Tier 1 Field Study: ***Are concentrations of PCDD/F in biota samples from the RMA greater than those in the same species collected from the selected off-post reference locations?***

Text Reference	American Kestrel Decision	Great Horned Owl Decision	Overall Terrestrial Species Decision
V.B.1	YES	YES	YES
V.B.1	YES	NO	YES
V.B.1	YES	Inconclusive	YES
V.B.1	Inconclusive	YES	YES
V.B.1	NO	YES	YES
V.B.2	NO	Inconclusive	Inconclusive
V.B.2	Inconclusive	NO	Inconclusive
V.B.2	Inconclusive	Inconclusive	Inconclusive
V.B.3	NO	NO	NO

[a] Text references are from BAS (2000). *Rocky Mountain Arsenal Dioxin/Furan Tier I Field Study Sampling and Analysis Plan*.

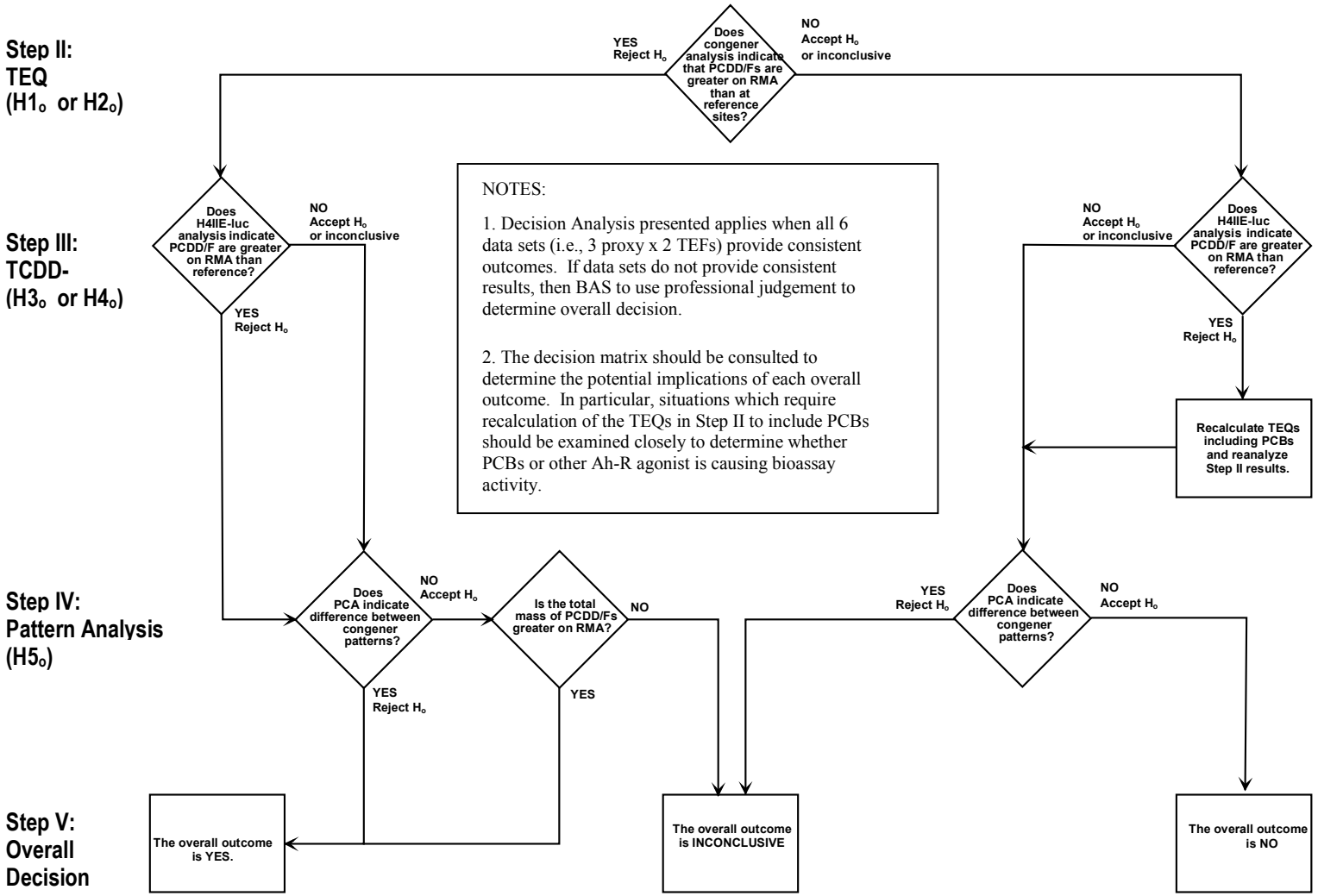
Table D. Decision Matrix for Carp Eggs to Support the Evaluation of PCDD/Fs as COCs at the RMA

Column 5 addresses the general question for this Tier 1 Field Study: ***Are concentrations of PCDD/F in biota samples from the RMA greater than those in the same species collected from the selected off-post reference locations?***

Step I: Data Usability	**Step II: TEQ ($H1_o$)**	**Step III: TCDD-EQ ($H3_o$)**	**Step IV: Pattern Analysis ($H5_o$)**	**Overall Outcome**
Evaluated	Reject $H1_o$	Reject $H3_o$	Use to determine principal components	YES
Evaluated	Reject $H1_o$	Inconclusive	Reject $H5_o$	YES
Evaluated	Inconclusive	Reject $H3_o$	Reject $H5_o$	YES
Evaluated	Reject $H1_o$	Inconclusive	Accept $H5_o$	Inconclusive
Evaluated	Inconclusive	Reject $H3_o$	Accept $H5_o$	Inconclusive
Evaluated	Inconclusive	Inconclusive		Inconclusive

Figure 6. Flowchart of Overall Decision Procedure for American Kestrel Eggs and Great Horned Owl Livers to Support the Evaluation of PCDD/Fs as COCs at the RMA

Are concentra6tions of PCDD/F in biota samples from the RMA greater than those in the same species collected from the selected off-post reference locations?



4.0 DATA ACCEPTABILITYAND USABILITY

4.1 Laboratory Quality Assurance/Quality Control

Various EPA guidances discuss proper selection of samples and analyses of results for comparisons to background concentrations, particularly the 1992 Data Usability for Risk Assessment in Superfund Guidance. The 1998 Guidance for Data Quality Assessment, EPA QA/G-9, and the 1999 Requirements for Quality Assurance Project Plans for Environmental Data Operations, EPA QA/R-5, are also good sources for information on making use of Data Quality Objectives along with establishing study criteria for acceptable field procedures and laboratory analytical performance. These guidances provide support to attain minimal required criteria for precision, accuracy, representativeness, completeness, and comparability (PARCC) of data. The SAP and its Decision Procedure, summarized in **Appendix** B, address the following processes.

Given the aim of this study and the assumption that background samples from off-post reference areas and/or times are collected to be as similar as possible except for exposure to site-released contaminants, then certain procedures for data management and analyses apply. For instance, outlier analyses can generally be performed on reasonably homogeneous off-post reference groups, but outlier analyses are usually not warranted for site data where the nature and extent of contamination has yet to be determined. The reason for this difference is that Tier I Field Study on-post outliers could represent discrete point sources and releases of contamination, rather than extreme variation of data outside certain standard deviations from the sampled mean. Elimination of on-post outliers at this screening stage of evaluation, before the nature and extent of contamination is known, could falsely eliminate areas with actual point sources or releases of COCs, and so it was (conservatively) not planned for this study. The possible downside is that one may include true outlier data in evaluations that skew results, weakening certain statistical test assumptions.

While all samples collected from on-post and off-post reference groups were initially considered by BAS scientists to be reasonably suitable for the intended use to represent contamination and exposure at their respective sites, there did develop obvious problems with emaciated on-post owls (a risk that one takes when using fortuitous screening specimens) that did require the rarely performed but justifiable outlier adjustments (discussed in detail in Section 4.3.2). The BAS also formed an internal workgroup that helped to audit and verify the reliability of the laboratory data, employing the assistance of laboratory chemists from EPA and from Army contractors, to examine and recommend uses of flagged sample results that did not fully qualify for intended use because of failure to meet predefined PARCC criteria as specified in the SAP. The QA/QC procedures were also conducted as specified in the Laboratory Quality Control Program (LQCP) requirements for MRI and for MSU. Usable data were compiled by the workgroup into standard spreadsheets for consistent later uses. The target MDL for 2,3,7,8-TCDD was 1 ppt for both laboratories.

Data that met acceptability criteria specified in the LQCP were categorized as “fully acceptable” and used in further analytical steps for TEQ and TCDD-EQ determinations in the tissue samples. Data that did not meet all criteria for acceptability in the LQCPs, but still met the minimal BAS's pre-defined usability criteria in the SAP, were classified as “usable” for further analytical steps in determining TEQ and TCDD-EQ; however, the significance of the LQCP short-comings were

described in uncertainty sections of BAS reports (see **Appendix** C1**, Table C1-1**). For example, flagged data were assessed for relative impacts on quantitative results, to ensure that proxy values did not artificially influence interpretations. Data that failed to meet any of the above criteria were reviewed by the BAS to decide how best to proceed with the less-certain usability of the data; e.g., recoveries were sometimes too far from 100%, or interferences caused MDLs for congeners to be too high. Some of these data were partially usable and sufficient for semi-quantitative analyses, rather than for quantitative statistical analyses to determine TEQs and TCDD-EQs.

Another aspect of data usability that was evaluated included the degrees of spatial and temporal *representativeness* of Tier 1 Field Study biota samples. Uncertainty existed for defining boundaries of the core population of kestrels, but the standard designation used by the USFWS Biomonitoring Program was used in this Tier I Screening Study for kestrel nest boxes located in sections 1, 2, 25, 35, and 36. An alternative would have been to measure dieldrin concentrations in kestrel eggs as an indicator to try to categorize birds with higher exposures to co-located PCDD/Fs on the RMA, but sample weights were inadequate. Kestrels have the advantage of a smaller home range that is roughly associated with spatially stratified nest box locations, and sufficient residency time to accumulate dieldrin in tissues of eggs, and therefore were assumed to be able to assimilate measurable PCDD/Fs attributable to the RMA. Owls, on the other hand, had wider foraging ranges that possibly included off-post locales, plus they were not spatially allocated over the RMA, which led to greater uncertainty about residency status and fractions of exposure attributable to the RMA. The owls also had limitations due to smaller sample numbers and wide variations in ages that could influence chronic uptake of bioaccumulating chemicals, partially overcome with the sampling of juveniles. Samples of 18 carp were collected on-post in the spring before spawning, but only two samples of carp were available from off-post lakes; however, this did not become a major limitation.

Selected off-post reference areas were anticipated to naturally vary somewhat in exposure and contamination, and thereby reflect a reasonably normal range of background concentrations of PCDD/Fs. However, because more than one off-post reference location was used, there existed a possibility of greater (perhaps statistically significant) differences in TEQ or TCDD-EQ and patterns occurring between different off-post reference locations than between the on-post versus off-post reference locations. There was also the possibility that outlying data points may occur within otherwise homogeneous groups of reference data (designated as greater than 2.5 standard deviations above the sample mean, or outside the 99th percentile of expected data). Divergent outliers were flagged and noted for their relative effect on the results; e.g., the highest kestrel egg TEQ concentration and relatively high owl liver concentrations were located in off-post reference samples.

4.1.1 Midwest Research Institute Chemical Analysis

The QA/QC procedures were conducted as specified in the SAP and in the LQCP requirements for MRI (MRI-5405-1,2.7). Initial draft results of analyses, which used a hybrid method to process (two extraction columns) and measure the 29 TCDD-like chemicals in fatty tissues, identified to the BAS workgroup that better understanding and definitions of laboratory flags were needed to properly evaluate and apply the data for comparisons in this Tier I Screening Study. A table of defined flags resulted, and is presented in **Appendix** C1**, Table C1-1**. The analytical laboratory performed in an excellent manner, based on results from random and blind

Interpretation and Use of Detection Limits

In many instances the concentrations of PCDD/Fs in the samples of this study were so low that they could not be detected by the trace-level analytical instrument. In this case, the laboratory reported the results as *non-detected* at the method detection limit (MDL) of the instrument. In other cases, the data may be qualified as *estimated* because the detection was lower than the level that the laboratory is confident in quantitatively reporting (the method quantitation limit or MQL), but the signal was greater than the MDL, or because the QA of the analytical procedure does not meet the QC criteria to report the actual value with enough confidence.

Scientists who use the data must decide how to properly apply data that are reported by a laboratory as non-detected or estimated. For this study, three sets of data were specified for the TEQ analysis, and two were specified for the TCDD-EQ analysis. It is common risk assessment practice to replace non-detect results with substituted proxy values, usually at ½ the MDL for the analyte. The purpose of specifying more than one data set to analyze is to evaluate the relative effect that the proxy values for non-detected analytes may have on the results; in effect this is a simplified sensitivity analysis.

For the **TEQ** analysis, the following three data sets were specified in the Decision Procedure:

TEQ_{FULL}: Full data set that includes non-detected and flagged data for each of the 29 congeners: substitute ½ the sample MDL for any sample result less than the MDL, and use the reported estimated value for each sample result between the MDL and the MQL

TEQ_{PAR}: Partial data set that includes flagged data: omit the non-detected analytes from the above full data set, and use the reported estimated value for each sample result between the MDL and the MQL

TEQ_{QUAN}: Fully quantitative data set that does not include proxy or disqualifying flagged data: include only data above the sample MQLs that are not annotated with a disqualifying flag

For the **TCDD-EQ** analysis the following two data sets were specified in the Decision Procedure:

TCDD-EQ_{FULL}: Full data set that uses the reported estimated value for each sample result between the MDL and the MQL (unanticipated, but can substitute ½ the MDL as proxy values for sample results that are less than the MDL)

TCDD-EQ_{QUAN}: Fully quantitative data set that does not include proxy nor flagged data: include only data above the sample MQLs that are not annotated with a disqualifying flag

While the Decision Procedure specifies the preferred use of the partial data set, these data were analyzed only in situations where different statistical outcomes were generated using the full and quantitative data sets. Only the full data set, as an exception to the Decision Procedure, using values above the sample MQL and ½ the MDL where concentrations were below the MDL, was used in pattern analyses. In principal components analysis (PCA), any congener that does not have a data value for every sample is eliminated from the analysis. Therefore, when a significant number of proxy values are present in a data set, this data reduction can result in the elimination of all the congeners from the PCA results. In addition, while proxy values can be misleading in risk assessment procedures, they can sometimes (depending upon analytical performance) provide valuable information for pattern recognition techniques. For example, a value less than the MDL can provide qualitative information on the concentration (with uncertainty) of that congener relative to other congeners greater than the MDL.

Data are presented as the full data set (TEQ_{FULL}) that contained flagged values and also included ½ the detection limit for those congeners that were below the MDL. The second data set presented (TEQ_{QUAN}) is a data set that contains no flagged data or data less than the MQL.

QC samples, which increased the confidence in the accuracy of trace-level (near 1 ppt TEQ) concentrations of the TCDD-like chemicals. Briefly, the laboratory reported sample-specific detection limits that were defined as a 4:1 signal to noise ratio, provided quantifiable results defined as a 10:1 signal to noise ratio, used a six-point calibration TEQ standard with internal calibration standards for the 29 congeners, plus used a corn oil matrix for blank control samples in each batch. The laboratory personnel provided full data sets of instrumental results with narrative reports. Army, EPA, and USFWS representatives from the BAS and the RMA conducted several audits at MRI, and generally good outcomes were found with corrective actions employed as necessary.

4.1.2 Michigan State University Bioassay Analysis

The QA/QC control procedures were also conducted as specified in the SAP and in the LQCP requirements for MSU (SOP # Table 3-4.1). Army and USFWS representatives also conducted a couple of audits at MSU, and generally good outcomes were found with corrective actions employed as needed. Procedures for the H4IIE-luc bioassay are presented fully in the SAP and in relevant standard operating procedures. The QA/QC plan divided within-assay procedures from matrix and sample procedures.

The within-assay QA/QC procedures ensured the acceptability of the assay results for individual samples. The prime QA/QC criterion for the bioassay is the determination of the assay EC50 (the dose of TCDD, which elicited half the maximal response) for the TCDD standard curve analyzed on each plate with each sample. The acceptability criterion for this parameter is the average for the QC lot plus 20%. Additional QA/QC procedures for each lot include the assay blank value and maximum luminescence for the TCDD standard curve. These two values were also used to generate a signal to noise ratio estimator for the assay by expressing the maximum standard value as a percentage of the blank values. A signal to noise ratio of 10 to 1 (standard greater than 1,000% of blank) is considered acceptable. These procedures ensure that the bioassay procedure truly reflects the concentration of TCDD-EQ in the prepared extract.

Sample and matrix QA/QC procedures were used to evaluate the acceptability of the entire sample preparation and assay procedure. These procedures included the analysis of laboratory blanks and spikes; matrix (chicken egg) blanks and spikes; selected sample duplicates (ensured reproducibility of extraction and assay procedures); nominal field standards (spiked with PCB-126); and pilot study samples to rule out dieldrin interactions. These procedures ensured that the assay results truly reflected the concentration of dioxin equivalents present in the samples.

4.2 Field Quality Assurance/Quality Control

4.2.1 Collection And Processing

As described earlier in this report, and available in the SAP and USFWS Biomonitoring Plan, samples were collected using standard operating procedures and sound scientific techniques.

4.2.2 Field Performance Evaluation (PE) Results

This study was also able to include 12 known standard samples for kestrel egg analyses, which included no, low, median, and high concentrations of a TCDD-like congener: PCB-126. These QC samples were prepared with clean quail eggs by an Army contract laboratory, and were submitted as blind and random duplicates (8 total), spiked with 0-, 10-, 100-, and 1000-ppt PCB-126; generating 0-, 1-, 10-, and 100-ppt TEQ when applying the WHO avian TEF of 0.1.

Another four random and blind samples of unspiked (naive controls) quail eggs were also included in the sample train. These samples worked well to help confirm the abilities of the two analytical laboratories to accurately and consistently measure trace-levels of PCDD/Fs in egg samples. From the results, it appeared that the chemical residue analysis by MRI was able to detect the lowest concentration with about 1 or less ppt TEQ, whereas the bioassay by MSU was able to detect down to the median concentration of about 10 or less ppt TCDD-EQ (both MDLs being adequate for this Tier I Screening Study).

4.3 Data Assessment

The data for this report are presented in **Appendix C** for analytical TEQs (C2 for American kestrel eggs, C3 for great horned owl livers, and C4 for carp eggs) and in **Appendix** D for bioassay TCDD-EQs. Nearly all of the data met the PARCC criteria for quality, with minor inconsequential exceptions for a few congeners with TEQ results or a few TCDD-EQ bioassay results in tissue samples. Since the use of proxy values had minimal impact on the quantitative differences in TEQs, the *full data set with all 17 PCDD/F congeners* was used for all statistical analyses (see **Appendix** E). The Decision Procedure in the SAP had originally called for truncating the quantitative data set for use in PCA (Principal Components Analysis) and pattern analysis; however, numerous non-detect values would have overly complicated or prevented the performance of the statistical test by using the more limited quantitative data set. The more qualitative data set with proxy values substituted for non-detect concentrations introduces more uncertainty in the pattern analysis.

4.3.1 American Kestrel Egg Data

The chemical residue analyses of PCDD/Fs and PCBs in eggs of kestrels proceeded quite well, considering that the kestrel egg samples were prioritized to be run first through the new hybrid method at MRI. This method was modified in an attempt to achieve lower detection limits of about 1 ppt of 2,3,7,8-TCDD, for better quantitation of trace-levels of PCDD/Fs in off-post reference tissues to statistically compare with potentially elevated concentrations from on-post tissues. Some problems were noted with elevated levels of congeners in the corn oil controls for batches run at MRI, and certain PCB analyses were occasionally problematic, but correction measures or adequate laboratory explanations were given to help the BAS workgroup properly interpret the data. The MSU bioassays appeared to perform acceptably, and clarification was provided to the BAS workgroup for fully understanding how the varied bioassay responses were processed during data reduction to normalize for comparable response endpoints with standard curves.

4.3.2 Great Horned Owl Liver Data

Because the owls were collected fortuitously from the RMA, there were factors that could not be controlled by sampling design. First, the ages of three of the dead owls collected on-post could not be determined; therefore, a third age class (unknown) was evaluated in statistical analyses. Tests were conducted by either treating these unknown age birds as adults (since most young first-year birds are identifiable), or by excluding these birds from the data set. Outcomes for both analyses are reported, and differences contribute to the range of uncertainty for owl results. Accurate determination of the age of the owls is significant, as the SAP specifies that tests be conducted to determine whether PCDD/F concentrations were different in the two age groups.

Second, and of more importance to the analysis, three of the four adult birds collected on-post were severely emaciated, presumably due to starvation caused (based on the probable diagnostic etiology) by dieldrin poisoning or infectious disease. Because of their lipophilic nature, TCDD-like chemicals have a high affinity for fatty tissues, and redistribution of body fat during a period of starvation would be expected to cause a redistribution of PCDD/Fs out of the primary fat stores to secondary storage tissues (e.g., liver and brain) in the bodies of these owls. Thus, emaciation would be expected to have a potentially large impact on the PCDD/F concentrations measured in the owl livers.

It was initially anticipated by the BAS that most, if not all, the adult owls would be reasonably representative of and accurate monitors for exposures to PCDD/Fs at the RMA by the indirect analysis of surrogate liver tissues, provided there was uniform uptake and deposition of lipophilic PCDD/Fs into the livers. Liver tissue was selected for analyses in owls since it is easier to process than carcasses, there was toxicity reference information to relate liver PCDD/F concentrations to toxic effects, more livers were available from fortuitous specimens than from carcasses (often consumed by necropsies for disease diagnosis), plus livers tend to accumulate PCDD/Fs (important for trace-level analyses). However, it was subsequently learned that the rank order and relative magnitude of the three highest liver concentrations coincided with only the three owls that were emaciated, which was considered to be an unlikely independent outcome and for which there was a precedence to suspect confounding causes may be producing falsely elevated concentrations in those livers. Furthermore, since all emaciated owls were found only on the RMA property, this could falsely implicate or exaggerate the likelihood of liver elevations being associated with a possible source of PCDD/Fs at the RMA.

4.3.2.1 *Literature Survey of Emaciation in Birds*

Two approaches were used by BAS scientists to determine what effect this confounding factor would have on the data analysis. First, a literature survey was carried out and is presented in **Appendix F**. This survey of the literature showed that no applicable data for the mobilization of PCDD/Fs in emaciated birds were available. However, data were available for the remobilization of some lipophilic organochlorines with similar biokinetic as well as physical and chemical properties. These studies, however, were not directly comparable to the RMA owls, since many of the test organisms were being fed high doses of the test compounds immediately before or during the emaciation phases of the acute exposures. This made interpretation of the studies difficult, as the alterations in tissue concentrations observed were influenced by both depuration of the recently ingested dose as well as mobilization of the chemicals from other body stores. The available studies suggested that a correction factor roughly between 1 and 5 might be applicable to adjust the liver concentrations to account for the effects of emaciation. However, it was the opinion of the BAS that this correction factor could not be used without further supporting site evidence.

4.3.2.2 *Whole Body Analysis and Liver Concentration Adjustment*

To assess the possible effects of emaciation in owls from the RMA on their PCDD/F concentrations in livers, a subset of adult owls was obtained for which most of the whole body parts were intact, and they were analyzed for PCDD/F burdens (mass) on a whole body basis. No carcasses of juvenile owls were available from USFWS archives to perform similar analyses on younger owls. Whole body concentrations of PCDD/Fs in available carcasses from adult emaciated owls were used to derive a site-specific correction factor for the TEQ concentrations

in the liver samples. The correction factor was used to ultimately adjust liver concentrations downward, so that the emaciated owl results would more closely approximate actual liver TEQ concentrations if the owls had not been emaciated. Without this adjustment, defensible statistical tests could not be validly conducted on adult owls. The main reason for this is that the sample groups were not comparable as a result of some owls being severely emaciated while others were not (which confounded the results by creating falsely elevated PCDD/F concentrations in the emaciated owls).

Results of tissue fat analyses showed that the average lipid concentrations in the emaciated birds were low at 2.9% (range 2.2 to 3.0) in liver and 1.5% (range 0.9 to 2.3) in muscle, compared to the higher 7.4% (range 2.5 to 17.2) in liver and 7.9% (range 2.4 to 13.3) in muscle of non-emaciated adult birds that were collected from off-post reference locations (see **Table 1**).

Table 1. Estimated body burdens of TCDD-like chemicals as 2,3,7,8-TCDD TEQs in selected adult owl carcasses

Specimen	Carcass Weight[1] (g)	Carcass Lipid (%)	Carcass TEQ (pg/g)	Carcass Mass (pg)	Liver Lipids (%)	Liver TEQ (pg/g)	Liver[2] Mass (pg)	Total Body Burden[3] (pg)	Liver:BW Mass Ratio (%)
On-Post Emaciated Owls									
96FGH002	631	0.9	17	10729	2.2	399	7,461	18,191	41%
96FGH007	728	2.3	130	94352	2.7	2360	51,448	145,800	35%
96FGH017	847	1.5	28	23334	3.0	594	16,394	39,729	41%
Off-Post reference Non-Emaciated Owls									
96RFGH01	769	13.3	8	6490	17.2	10	229	6,718	3%
96RFGH03	896	6.6	30	26618	4.6	122	3,210	29,828	11%
96RFGH05	700	8.0	11	7934	3.0	12	276	8,210	3%
96RFGH07	975	9.4	24	23829	2.5	12	359	24,188	1%
96RFGH12	702	2.4	16	11220	9.9	24	578	11,798	5%

[1] Carcass weights are the net homogenized yield of soft tissues, assumed to contain nearly all PCDD/Fs
[2] *Estimated* from mean liver: body weight (**pre**-homogenized) ratio of 0.026 in birds (Barton and Houston 1996)
[3] Calculated by summing the carcass (missing the livers) and estimated liver burdens, with rounding errors
Note: The carcass of one of the adult on-post birds was not available for whole body analysis.

Differences in mean lipid content measured in liver samples were not statistically significant, due to low sample numbers and the larger variations in lipid contents of off-post reference owls, even though all three on-post owls' liver lipid percent were very similar and at the low end of the range found for non-emaciated off-post reference owls. Differences measured in carcass lipid content were significant for reduced amounts of body fat in the emaciated on-post owls (Mann-Whitney U-test, $p < 0.05$). The differences in carcass lipid measurements suggest that a significant reduction of body fats had occurred, which typically would mobilize any previously stored fat-seeking chemicals. These chemicals would not be expected to be eliminated significantly from the body but rather redistributed to other lipid-containing tissue (e.g., brain and liver).

The total body concentrations estimated for the available on-post and off-post reference owls were compared using the Mann-Whitney U-test, and they were not significantly different ($p = 0.18$). The Mann-Whitney U-test is a non-parametric method that ranks data and then

performs analysis on the relative ranks, so that no assumptions of normality or homogeneity are required to validly analyze the data. However, unadjusted liver TEQ concentrations and liver body-mass ratios of TEQs were significantly ($p < 0.05$) elevated in emaciated owls as compared to the non-emaciated off-post reference owls.

The correction factor for elevated liver concentrations in emaciated owls could be derived by several means, based upon various assumptions and approaches. The BAS scientists considered it best to calculate the normally expected distribution ratios of the body burdens of TEQs between the estimates for liver and the whole body in off-post reference adults. Since liver weights in the fortuitous owls were unavailable, having been used up for dieldrin analyses, an avian mean % liver to body weight was substituted to estimate liver weights. The correction factor was applied to the measured liver concentrations in emaciated owls to calculate an expected "pre-emaciated" liver TEQ concentration. Specifically, the average percent of PCDD/F mass as TEQs in liver versus whole body for the non-emaciated owls was about 5%, while the average mass in the unadjusted liver versus whole body for the emaciated owls was about 40%. Therefore, a conservatively low adjustment of an 8-fold decrease was used to normalize PCDD/F concentrations in livers from emaciated owls (**Table 2)**.

Table 2. Raw and Adjusted* TEQ Concentrations (ppt) in Livers of Great Horned Owls

Sample	Carcass TEQ	Measured Liver TEQ	Adjusted* Liver TEQ
On-Post Emaciated Owls			
96FGH002	17	399	50
96FGH007	130	2,360	295
96FGH017	28	594	74
Off-Post Reference Non-Emaciated Owls			
96RFGH01	8	10	--
96RFGH03	30	122	--
96RFGH05	11	12	--
96RFGH07	24	12	--
96RFGH12	16	24	--

* Adjusted TEQs were calculated by dividing the measured values by 8 as described in the text.

This approach was deemed to likely be a more accurate option, but also conservative (minimal adjustment), since other approaches (e.g., only considering ratios of *concentrations* in liver to whole body) produced greater possible downward adjustments in emaciated liver TEQs. The rationale for using this method is based on the fact that PCDD/Fs are only slowly eliminated from the body; therefore, the whole body burden of PCDD/Fs in the owls would be essentially the same before and after emaciation. As a result, the "original" concentration of PCDD/Fs in the liver could be reconstructed as a function of the whole body TEQ mass along with the liver and body weights, while considering the relative changes in liver and body lipid contents. The resulting value of 8 for the adjustment factor accommodated for the higher partitioning of TEQs in the livers of emaciated owls. This factor was decided upon by the BAS after close examination of all the data and the application of best scientific judgment, while employing a degree of conservative bias to ensure that the probability of a false negative conclusion being reached was not increased unjustifiably.

The determined adjustment factor of 8-times was also applied to the data for the bioassay results before statistical analysis, as the same factors leading to greater concentrations of the chemicals

responsible for the TEQ should also apply to most of the chemicals that are active in the bioassay. While some less persistent chemicals such as polycyclic aromatic hydrocarbons (PAHs) are active in the bioassays, they are relatively easily metabolized and so do not generally accumulate to the same degree as PCDD/Fs. PAHs would not be expected to concentrate as much in the liver as do TCDD-like chemicals during lipid mobilization, and PAHs tend to be oxidized or conjugated by the liver and eliminated from the body faster. This suggests that using the concentration factor of eight for TCDD-EQ could be an overestimate of the concentration factor for the bioassay measurement of TCDD-like activity.

It was also noted that the off-post reference specimen 96RFGH03 had the greatest ratio of liver to whole body concentrations of TEQ in the off-post reference group, suggestive of some remobilization of TCDD-like chemicals to the liver; however, this ratio was still about one-fourth that of individuals from the on-post group. While this bird did not appear to be clinically emaciated, its liver and body lipid contents were greater than any of the emaciated birds, ranking third of five for liver and fourth of five for body lipid within the off-post reference group. This individual may be an outlier due to several possible causes, including effects of age, disease, and nutrition, etc. Even so, there appears to be a consistent demarcation of increased partitioning of body burdens of PCDD/Fs into the livers of emaciated owls.

4.3.3 Carp Eggs Data

The results for carp eggs were all very low in terms of exposure and risk, being near the trace-level detectable concentrations for PCDD/Fs. No substantial problems were noted with the chemical analyses of the samples. Even though there were only two samples of carp eggs from off-post to represent off-post reference area exposures, the concentrations were so low as to be judged highly unlikely to pose any threat of over-exposure to possible PCDD/Fs from the RMA. Therefore, the BAS recommended that the aquatic portion of the dioxin study was sufficiently informative to rule out possible excess risks.

5.0 ANALYTICAL AND STATISTICAL RESULTS

In this section of the report, concentrations are presented for TEQ as determined by chemical analyses and for TCDD-EQ as determined by bioassay. The Decision Procedure was used to determine whether concentrations of dioxins and furans were greater on-post than off-post. If so, then the possibility would exist that PCDD/Fs should be considered COCs at the RMA. While the pre-defined Decision Procedure was followed as closely as possible, there were points at which some divergence from the procedure was unavoidable due to the nature of the data set collected. Where these divergences were required, the scientific reasons for them were discussed.

To keep this section of the report brief, only summaries of the statistical results are reported. The complete data reports for the chemical analysis are included as **Appendix C** (C2 for American kestrel eggs, C3 for great horned owl livers, and C4 for carp eggs), while the data reports for the bioassay results are included as **Appendix** D, and the outputs from the statistical tests are provided as **Appendix** E.

5.1 American Kestrel Eggs

The collection of American kestrel eggs from nest boxes that were arrayed in a grid pattern on the RMA provided the most systematic means of screening samples for potential exposure of wildlife to PCDD/Fs which might be present. The collected eggs represent a set of uniform tissues with a defined subchronic exposure period. In addition, the systematic arrangement of the nest boxes provided the most spatially representative data set, with similar sample sizes collected in the core and periphery of the RMA, as well as from off-post reference locations.

5.1.1 Samples Collected

In the 1998 field season, 11 eggs were collected from the core area of the RMA, 19 from the periphery, and 16 samples were collected from off-post reference locations near the RMA (**Table 3**). This set of samples also included 12 quail eggs that were used as a random blind QC samples.

Table 3. American kestrel eggs collected for the Tier I Field Study

On-Post			Off-Post
Total (O)	Core[a] (C)	Periphery (P)	Reference (R)
30	11	19	16

[a] The core population area (C) was defined per the USFWS Biomonitoring Program consisting of birds that potentially nest or feed in RMA Sections 1, 2, 25, 26, 35 and 36 (12 nest box locations designated NW02, NW06, NW07, NW11, NW12, NW25, NW26, NW30t, NW31, SE35, NE35, and NW35). The other possible definitions for the core population, as described in the Decision Procedure, were not used because no alternative spatial pattern of contamination was observed. Note that only 11 of the 12 nest boxes were occupied resulting in only 11 samples collected.

5.1.2 TEQ Results

Mean concentrations of TEQ measured in American kestrel eggs ranged from 10.8 to 22.4 ppt TEQ for the 17 dioxin and furan congeners for the three comparison groups (R, P, C), when the full data set (TEQ_{FULL}) was used (**Table 4**). In this data set, a value equal to ½ the MDL was assigned for concentrations less than the MDL, so that each of the 17 congeners had an assigned value. Using the quantitative data set (TEQ_{QUAN}), where results less than the MDL and disqualified flagged data were omitted, the average concentrations ranged from 8.3 to 18.3 ppt. The median (half the results above and half below) values for these measurements were considerably lower than the arithmetic mean values, indicating that the data set was skewed left with most values near the median and only a limited number of measurements at greater concentrations. Concentrations of TEQs are also plotted in **Figure 7**.

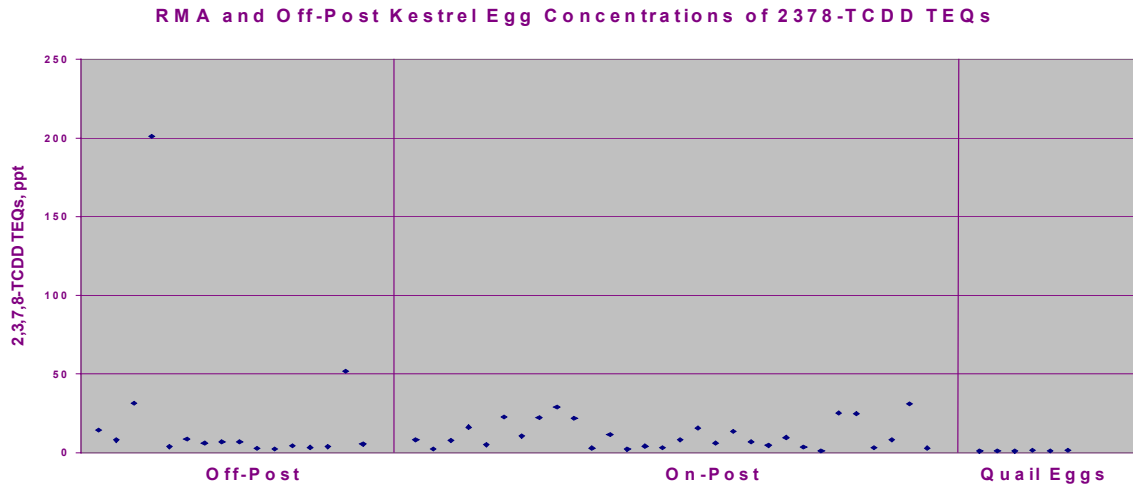
To assess the impact of frequencies and magnitude of proxy values on the data set, as specified in the Decision Procedure, the contribution of TEQ values from proxy values in the full set (with higher concentrations) compared to the quantitative set (with lower concentrations) of data was evaluated for each sample. To be within the acceptability criteria of the Decision Procedure, the relative TEQ contributed by the proxy values had to be less than 50% of the total TEQ for more than 50% of the samples. The contribution to TEQs by proxy values was less than 50% for 10 of 16 off-post reference area, 14 of 19 peripheral area, and 8 of 11 core area samples; therefore, the

Table 4. Summary of TEQ concentrations (ppt) measured in American kestrel eggs collected in the core (C) or periphery (P) areas of the RMA or from off-post reference (R) locations

Measure	Group	Number	TEQ ppt			
			Mean	Minimum	Maximum	Median
TEQ_{FULL}	R	16	22.4	2.2	201.0	5.7
	P	19	10.8	2.1	31.0	7.8
	C	11	11.8	1.1	29.1	8.2
TEQ_{QUAN}	R	16	18.3	0.1	177.5	2.9
	P	19	8.3	0.0	27.0	5.0
	C	11	8.8	0.1	27.3	4.5

decision criteria were met, since proxy values did not have an excessive influence on TEQs. As would be expected, the contribution of the proxy values was greatest for samples with small TEQ concentrations (TEQ less than 10 ppt), because these samples contained the greatest proportion of results less than the MDL and were more variable near the detection limit.

Figure 7. Plot of TEQs observed in eggs from American kestrels collected on-post at the RMA and from off-post reference areas, along with quail egg blank controls



5.1.2.1 *Distribution Analysis*

The frequency distribution of concentrations of TEQs in kestrel eggs was tested to determine if TEQs followed a normal distribution, using probability plots and the one-sample Kolmogorov-Smirnov test (**Table 5** and **Figure 8**). The Kolmogorov-Smirnov test with Lillifors' distribution produces a standardized distribution of the data and compares that distribution to that expected for a normal distribution. A significant ($p < 0.05$) test result indicates that the data distribution is significantly different from a normal distribution (**Table 5**). If the data diverge significantly from a normal distribution, the non-parametric rather than the parametric statistical methods should be used; or, other normality tests can also be run to confirm or refute close findings. The data for kestrels were not normally distributed but were when logarithmically transformed, allowing use of the Student's t-test

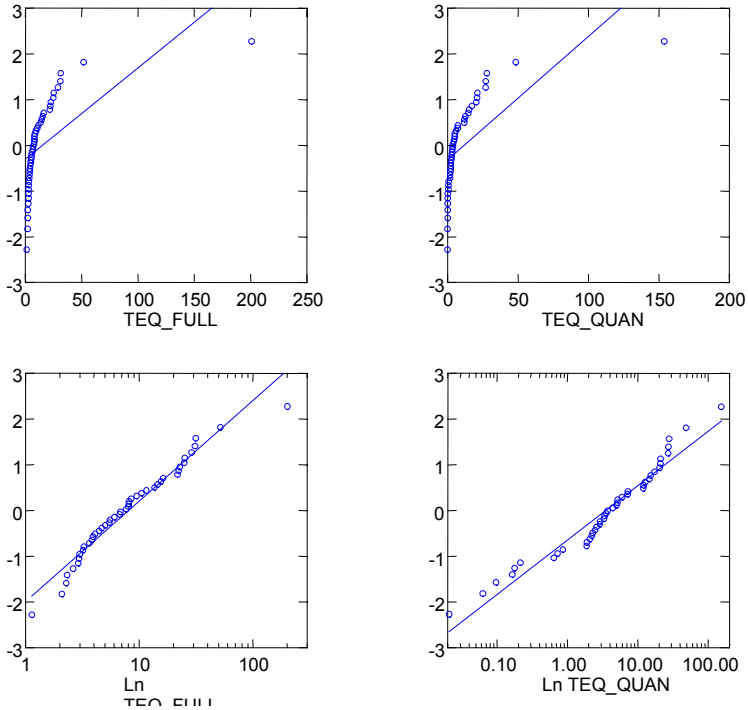
only for transformed data. (Note: All logarithmic transformation of data was carried out using natural logarithms [ln].)

Table 5. Probability (*p*) of deviation from normality for untransformed and natural ln-transformed data for American kestrel eggs using the Kolmogorov-Smirnov test

Measure	Mean	Standard Deviation	Deviation from Normality (*p*)
TEQ_{FULL}	15.1	29.9	< 0.001
TEQ_{QUAN}	11.9	27.0	< 0.001
ln TEQ_{FULL}	2.1	1.0	0.72
ln TEQ_{QUAN}	1.3	1.8	0.19

The distributions of the TEQ and TCDD-EQ data were also assessed for normality by producing probability plots (**Figure 8**).

Figure 8. Normal probability distributions for untransformed (upper) and ln-transformed (lower) TEQ concentrations (ppt) for American kestrel



These diagrams plot the measured values of the data points against the values that would be expected if the data exhibited a normal distribution. If the data can be described by a normal distribution, then the data should closely fit a straight diagonal line. Deviations from normality implies that the data cannot be analyzed using statistical procedures such as the Student's t-test or analysis of variance (ANOVA) because these procedures are based on the assumption that the data are normally distributed.

5.1.2.2 *Statistical Analysis*

To determine if there was a statistically significant difference between concentrations of TEQ for kestrels, a Student's t-test (with only a 1-tail criteria applied to test if on-post results exceed off-post reference results) was performed using ln-transformed data, which were compared using either separate or pooled variances (**Table 6**). This t-test and subsequent tests were conducted using two methods to analyze for homogeneity of variance, because no preferred method was stated in the Decision Procedure. Neither the TEQ_{FULL} nor the TEQ_{QUAN} data set showed a significant difference ($p < 0.05$) between on-post and off-post reference sample populations.

Table 6. Statistical significance (*p*) of mean differences by Student's t-tests on ln-transformed data for comparing TEQ concentrations (ppt) between on-post (O) and off-post reference (R) American kestrel eggs (using pooled and separate variances)

Data Set	Group	Number	Mean (ppt)	Standard Deviation	*p*	
					Separate	Pooled
TEQ_{FULL}	R	16	2.1	1.2	0.90	0.89
	O	30	2.1	0.9		
TEQ_{QUAN}	R	16	1.0	2.1	0.32	0.26
	O	30	1.6	1.3		

In addition to the Student's t-test, an ANOVA followed by Dunnett's test was conducted to allow valid multiple comparisons of the three distinct sample populations (**Table 7**). During the ANOVA procedure some values were identified as "statistical outliers." The concentration of TEQ in sample AKEG012 was the greatest value for kestrels in the study and was identified to be a statistical outlier. This sample was collected near Aurora reservoir; two other samples from this location did not show elevated concentrations of TEQ. The AKEG012 sample also contained the greatest concentration of TCDD-EQ as determined by the H4IIE-luc assay. For the full data set, no significant differences ($p < 0.05$) were detected between either RMA sample mean (core or periphery) and the mean of the off-post reference samples, either with or without the single statistical outlier (AKEG012) removed in the ANOVA.

Table 7. Statistical significance (*p*) of mean differences of TEQ concentrations (ppt) by ANOVA followed by Dunnett's test for ln-transformed data of core (C) or periphery (P) American kestrel eggs compared to the off-post reference (R) samples

Data Set	*p*	
TEQ_{FULL}	Core	Periphery
+ outlier	0.50	0.49
- outlier	0.39	0.43
TEQ_{QUAN}	0.42	0.17

5.1.2.3 *Power Analysis*

Statistical analyses were determined to be significant if the probability of a false positive result was less than 5%. That is, if α is less than 0.05, there is only a 5% chance of concluding that the means are different when in fact they are not different. A second statistical comparison parameter is termed beta (β) and represents the risk of a false negative conclusion, concluding the means are not different when in fact they are. The ability to avoid false negative conclusions indicates the statistical power of the test. If the statistical power ($1-\beta$) is small (i.e., β is great), then there is a relatively great risk of wrongly concluding that the means being tested are not different. To avoid these false conclusions, the BAS adopted the commonly used benchmark of requiring β to be less than 0.2 (i.e., statistical power [$1-\beta$] greater than 0.8). The power of a test is determined by the sample sizes, differences in the means, and variability within the sample populations. For each assessment used in the current study, a power analysis was carried out to determine whether the values of β and the statistical power were sufficient to provide a valid test that supports the conclusion that significant differences exist between sample means. If statistical power was too small, then the results of the comparison were determined to be inconclusive.

While the statistical power of the test, based on measured concentrations in kestrel eggs, was insufficient (**Table 8**), this may simply have been because there was no difference between the means. As indicated in the Decision Procedure, power analysis was also conducted to determine whether the study design had sufficient power to detect a difference of at least 15 ppt between the off-post reference and on-post sample populations. This evaluation was made by adding 15 ppt to each of the individual measurements. Since this process alters the standard deviation of the sample, the test was conducted using both the original greater standard deviation and the standard deviation generated from the modified data. Use of the unmodified standard deviation is equivalent to assessing the difference by simply adding 15 ppt to the mean of the on-post samples. In both cases, the study design had sufficient power to detect differences of 15 ppt or greater between the on-post and off-post reference sample populations for kestrel eggs (**Table 8**).

Table 8. Statistical significance (*p*) and power analysis ($1-\beta$) of mean differences in TEQ concentrations (ppt) between ln-transformed data for on-post (O) and off-post reference (R) American kestrel eggs

Measure	Group	Number	Mean (ppt)	Standard Deviation	*p*		Beta (β)	Power ($1-\beta$)
					Separate	Pooled		
TEQ_{FULL}	O	30	2.1	0.9	0.90	0.89	0.96	0.04
	R	16	2.1	1.2				
TEQ_{QUAN}	O	30	1.6	1.3	0.32	0.26	0.73	0.27
	R	16	1.0	2.1				
15 ppt difference								
TEQ_{FULL}	O	30	3.2	0.3	0.002	< 0.0001	0.02	0.98
	R	16	2.1	1.2				
TEQ_{QUAN}	O	30	3.1	0.3	0.001	< 0.0000	0.01	0.99
	R	16	1.0	2.1				

5.1.3 TCDD-EQ Results

Quantifiable concentrations of TCDD-EQs were found in only five of 46 American kestrel egg samples. Mean concentrations of TCDD-EQ in samples of kestrel eggs ranged from 3.0 to 15.5 ppt TCDD-EQ when using the full data set, while mean concentrations ranged from 0.6 to 13.6 ppt TCDD-EQ when the $TCDD\text{-}EQ_{QUAN}$ values were used (**Table 9**). As with the TEQ data, the measurements appeared to be skewed with the median concentrations being less than the mean concentrations, probably again due to the large number of non-detected results (results less than the MDL).

Table 9. Summary of TCDD-EQ concentrations (ppt) in American kestrel eggs collected in the core (C) or periphery (P) areas of the RMA or from an off-post reference (R) location

Measure	Group	Number	TCDD-EQ (ppt)			
			Mean	Minimum	Maximum	Median
$TCDD\text{-}EQ_{FULL}$	R	16	15.5	0.5	122	2.5
	P	19	3.0	0.5	11	2.5
	C	11	7.6	0.5	62	2.0
$TCDD\text{-}EQ_{QUAN}$	R	3	13.6	NA	122	NA
	P	1	0.6	NA	11	NA
	C	1	5.6	NA	62	NA

5.1.3.1 *Distribution Analysis*

The data were tested for normality by the Kolmogorov-Smirnov test (**Table 10**) and probability plots (not shown). The data were not normally distributed but were log-normally distributed with the exception of the sample set from the off-post reference areas. Because off-post reference samples were collected from more than one location, this is not surprising and the minor deviation from normality was not considered critical to the analysis. Therefore, ln-transformed data were used in the Student's t-test to determine the significance of any differences. Since the number of samples in which TCDD-EQ was detected was small, two detections in on-post samples, and three detections in off-post samples, distribution functions could not be calculated or compared.

Table 10. Probability (*p*) of non-normality for TCDD-EQ concentrations (ppt) in American kestrel egg samples as determined by Kolmogorov-Smirnov test
(R = reference, P = periphery, C = core, ln = log normal)

Group	Measure	*p*
R	$TCDD\text{-}EQ_{FULL}$	< 0.000
	ln $TCDD\text{-}EQ_{FULL}$	0.03
P	$TCDD\text{-}EQ_{FULL}$	< 0.000
	ln $TCDD\text{-}EQ_{FULL}$	0.40
C	$TCDD\text{-}EQ_{FULL}$	< 0.000
	ln $TCDD\text{-}EQ_{FULL}$	0.22

5.1.3.2 *Statistical Analysis*

The same statistical analyses were conducted for TCDD-EQs measured in the H4IIE-luc bioassay as those conducted for concentrations of TEQs. In the case of the bioassay data only

one data set was used. The quantitative data set, from which values less than the MDL were removed, was not used in this analysis because the number of samples greater than the MDL was only five. Concentrations of TCDD-EQs in kestrel eggs were not significantly different between eggs collected on the RMA and eggs collected from off-post reference locations (**Table 11**).

Table 11. Statistical significance (*p*) and power analysis of mean differences by Student's t-test between ln-transformed concentrations (ppt) of TCDD-EQ in on-post (O) and off-post reference (R) American kestrel eggs

Measure	Group	Number	Mean	Standard Deviation	*p*		beta (β)	Power (1-β)
					Separate	Pooled		
$TCDD\text{-}EQ_{FULL}$	O	30	0.8	1.1	0.231	0.18	0.998	0.002
	R	16	1.3	1.5				
15 ppt difference								
$TCDD\text{-}EQ_{FULL}$	O	30	2.9	0.3	NA	NA	0.00001	0.999
	R	16	1.3	1.5				

5.1.3.3 *Power Analysis*

A power analysis was conducted to determine whether the lack of significant difference between on-post and off-post reference sample populations was due to a lack of statistical power. There was insufficient power to distinguish between the means of concentrations of TCDD-EQs on-post and off-post reference as measured. However, there was sufficient statistical power to measure a difference of 15 ppt between the groups (**Table 11**).

5.1.4 Pattern Analysis

To determine whether patterns of contaminants were different between on-post and off-post reference samples, two pattern analysis techniques were planned. The first approach was PCA, which provides a visual interpretation of patterns of complex chemical mixtures but does not permit rigorous statistical hypothesis testing. The second technique was profile analysis, which permits statistical testing of hypotheses.

Because of the nature of the data sets collected, some variations from the Decision Procedure were necessary. Variations were also necessary to account for situations not anticipated in the SAP. The main variation was that the data were standardized before analysis. The standardization procedure takes the data for each congener and sets the average to zero and the standard deviation to 1. Standardization is extremely useful when performing PCA on data for PCDD/Fs due to the wide range in absolute concentrations of the different congeners. For example, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is typically in the 0- to 5-ppt concentration range while octachlorodibenzodioxin (OCDD) is usually in the 100 to 1,000 ppt range. Standardization removes all influence of the disproportionate absolute concentrations and gives each congener equal weighting in the analyses. Since all congeners are given equal weighting, this procedure also removes the need, as indicated in the Decision Procedure, to assess the impacts of congeners with low toxicity equivalent factor (TEF) values (< 0.0001). The inclusion of these congeners is important in the analysis, because the more chlorinated congeners that have low TEF values are informative indicator compounds for specific sources of PCDD/Fs.

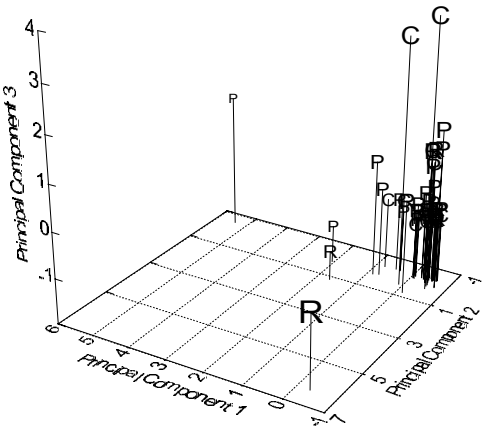
For the PCA on the kestrel data set only the full data set could be effectively used due to the large number of values less than the MDL. In PCA, the loss of a data value for a congener in a single sample would mean that the specific congener could not be used in the analysis. While some statistical methods can be used to approximate a value for these missing data, the methods vary in their applicability to different data sets.

For PCDD/F data, it is the most acceptable scientific and regulatory procedure to use ½ the MDL as was done for the full data set. While it is possible that use of such surrogate values could influence the outcome of the PCA, a systematic difference (i.e., a consistent difference in detection limits for either on-post or off-post reference results) would be required between the two sample groups. Because tissue samples were submitted to laboratories in a blind (coded) and random manner, with on-post and off-post specimens mixed in submission order, the possible bias with disparate MDLs should not exist. Furthermore, it is not considered possible that such influence would sufficiently alter the results as to mask the existence of a distinctive congener pattern. Assessment of the data demonstrated no such consistent differences (data not shown). Therefore, such influence by substituted proxy values for non-detected congener concentrations would also not be sufficient to alter the overall Decision Procedure for the kestrel egg data.

The PCA was performed using the full data set that contained all quantifiable data and ½ the MDL for concentrations below the MDL. For PCA, the data were first standardized to "z scores." These values are calculated by setting the mean and standard deviation of the data for each variable to values of zero and one, respectively. The PCA procedure then combines all the variability in the data set and generates a series of orthogonal axes to describe this variability. With this analysis, samples that have a similar congener profile will ordinate or cluster together to form a distinct group. In the case of organic contaminant analyses, 'background' samples generally form a cluster near the center of the distribution and those samples with a common profile of elevated concentrations from a point source release would be expected to form distinct clusters away from this center.

The results of the PCA for American kestrel eggs are presented in **Figure 9**. This figure is dominated by a close grouping of both off-post reference and on-post samples, which suggests that the patterns in these eggs represent a background profile of PCDD/Fs to which all birds in the study are exposed. This grouping may, to some extent, be the product of a significant number of non-detect values that can cause clustering, especially at trace-levels of concentrations where analytical noise is greater than found for results above the MQL. Standing apart from the central cluster are several samples from both off-post reference areas and from on-post. The samples that are separate from the central cluster do not form a separate grouping but are distributed around the graph. This outcome indicates that there is not a distinct pattern of PCDD/F congeners common to these samples as would be expected if a point source of contamination existed on the RMA.

Figure 9. PCA results for PCDD/F profiles in American kestrel eggs
(C = core, P = periphery, R = reference)



The lack of a pattern of contamination indicates the profile analysis would not be appropriate. Profile analysis is used as a quantitative statistical analysis of the difference between two groups of samples sharing two distinct contaminant profiles. As there is no unified pattern distinguishing the outlying samples in this analysis; profile analysis cannot be conducted.

5.1.5 Conclusions for American Kestrel Eggs

The decision matrix below (excerpted from **Table A** in **Section 3**) results in an outcome of *NO* for the American kestrel egg data. Concentrations of TEQ in kestrel eggs are not greater in on-post samples than in samples collected in off-post reference areas. There is no statistically significant difference in the average concentration of TEQ in on-post kestrel eggs than in the off-post kestrel eggs, thus the null hypothesis (H_o) is accepted for this step. Similarly, concentrations of TCDD-EQ are not significantly greater in on-post kestrel eggs. Statistical tests for these comparisons have sufficient power to detect the desired differences. Thus, the null hypothesis is also accepted for this step on TCDD-EQ results. Finally, PCA of the data indicate that there is no common pattern of PCDD/F congeners that distinguish on-post from off-post reference samples, leading to the acceptance of the null hypothesis.

Step I: Data Acceptability	Step II: TEQ ($H1_o$ or $H2_o$)	Step III: TCDD-EQ ($H3_o$ or $H4_o$)	Step IV: Pattern Analyses ($H5_o$)	Step V: BAS's Answer for Overall Decision
Yes	Accept H_o	Accept H_o	Accept H_o	NO

5.2 Great Horned Owl Livers

Great horned owls are of interest among the raptors because they are also resident species of the RMA and surrounding areas, although with wider home and foraging ranges (miles) in general than found for nesting American kestrels (mostly within a mile). Additionally, great horned owl specimens were available from the USFWS fortuitous specimen program for use in this Tier I Screening Study of exposure to and uptake of PCDD/Fs from potential RMA sources.

5.2.1 Samples Collected

Great horned owls were collected fortuitously, mostly during 1996, and were used in this study. Specimens were assigned to one of three groups based on apparent age at death. Four adults, nine juveniles, and three owls of unknown (likely adults, since juveniles are distinctive, but still uncertain) age were collected from the RMA. The off-post reference group consisted of five adult and five juvenile owls.

Table 12. Great horned owls (GHO) collected for the Tier I Field Study

GHO Age	On-Post GHO (O)	Off-Post Reference GHO (R)
Adult (A)	4	5
Juvenile (J)	9	5
Unknown	3	0

5.2.2 TEQ Results

Average concentrations of TEQs were greatest in on-post adults and least in off-post juveniles. The data for all groups were characterized by large standard deviations as a result of the relatively great variability within the sample groups. The TEQ concentrations in owls are summarized in **Table 13**.

Table 13. TEQ concentrations (ppt) in great horned owl livers from on-post (O) and off-post reference (R) samples

(Emaciated owl TEQs were adjusted per Section 4.3.2.2)

	Group	Number	Mean	Minimum	Maximum	Median
TEQ_{FULL}						
Adult	O	4	157.2	49.8	295.1	140.0
Juvenile	O	9	31.5	1.9	98.4	22.0
Unknown	O	3	47.2	13.9	95.6	32.2
Adult	R	5	36.0	10.4	121.6	12.4
Juvenile	R	5	22.1	8.2	34.0	23.8
TEQ_{QUAN}						
Adult	O	4	156.2	49.1	294.0	140.9
Juvenile	O	9	29.9	0.7	97.8	20.5
Unknown	O	3	45.9	12.0	94.8	30.8
Adult	R	5	31.4	3.3	110.4	11.1
Juvenile	R	5	13.0	0.5	30.3	12.3

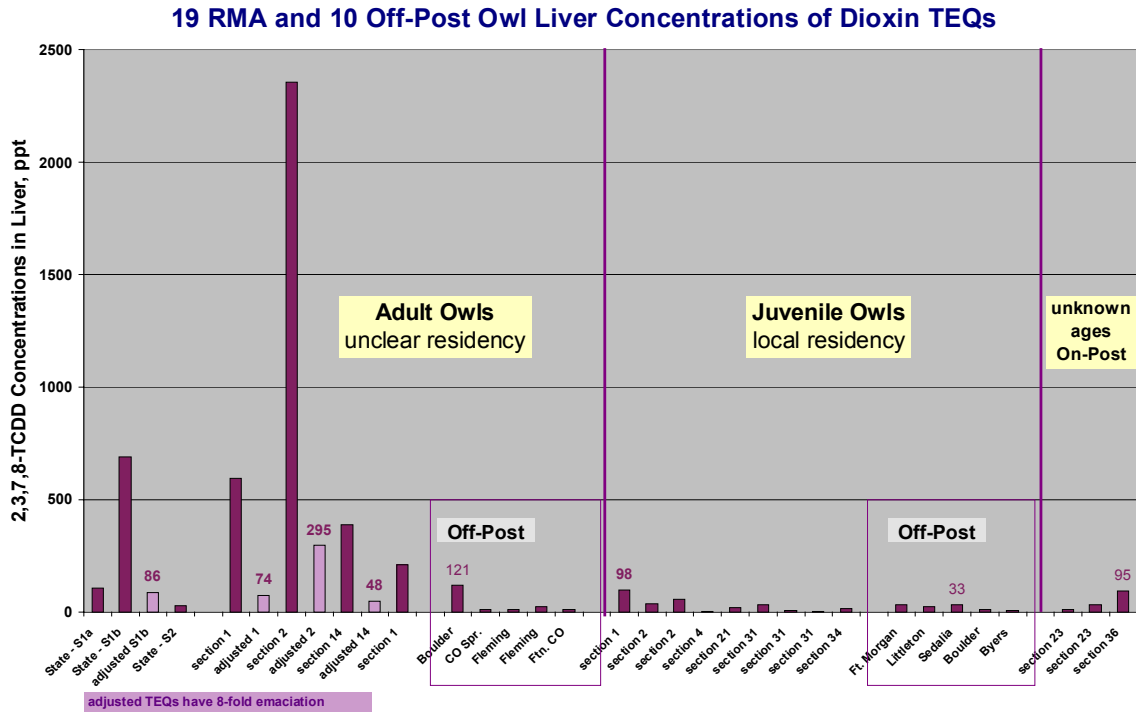
The owls were grouped into categories of adult, juvenile, and unknown age (**Figure 10**). When the owls of unknown age were excluded from statistical analysis, there was a significant difference ($p = 0.02$) between on-post adults and juvenile owls in both the full and quantitative data sets (**Table 14**). This difference between TEQs in younger vs. older owls began to disappear when on-post owls of unknown age were included as adults ($p = 0.05$) and was absent in off-post owls. Therefore, each age class of owls was analyzed separately.

Table 14. Statistical significance (*p*) of mean differences by Mann-Whitney U-tests comparing differences in TEQ concentrations (ppt) for different age groups of great horned owl liver samples
(A = adult, J = juvenile)

Group	Number	*p*	
On-post GHO	**(A, J)**	**TEQ_{FULL}**	**TEQ_{QUAN}**
Unknown age excluded	4, 9	0.02	0.02
Unknowns age as adults	7, 9	0.05*	0.05*
Off-post reference GHO	5, 5	0.75	0.60

*Significance (p) was not < 0.05, although close at $p = 0.0502$

Figure 10. Plot of TEQs observed in livers from great horned owls collected on-post and from off-post reference areas, categorized by ages



5.2.2.1 *Distribution Analysis*

The distribution of the owl data was tested using the Kolmogorov-Smirnov test with Lillifors distribution both before and after ln transformation (**Table 15**). Because of the TEQ differences between age class, each age class and location was tested independently as was the effect of including the unknown aged owls as adults. Of all the data sets, only the untransformed data for the adult off-post reference owls showed a significant deviation from normality.

Table 15. Significance (*p*) of Kolmogorov-Smirnov test for deviation from normal distribution for great horned owl liver data

(a *p* value less than 0.05 indicates a distribution significantly different from a normal distribution)

Group	N	Measure	*p*
On-post juvenile	9	TEQ_{FULL}	0.70
	9	TEQ_{QUAN}	0.44
	9	ln TEQ_{FULL}	0.88
	9	ln TEQ_{QUAN}	0.41
Off-post reference, juvenile	5	TEQ_{FULL}	1.00
	5	TEQ_{QUAN}	1.00
	5	ln TEQ_{FULL}	0.68
	5	ln TEQ_{QUAN}	0.49
Off-post reference, adult	5	TEQ_{FULL}	0.01
	5	TEQ_{QUAN}	0.01
	5	ln TEQ_{FULL}	0.17
	5	ln TEQ_{QUAN}	1.00
On-post, adult unknowns excluded	4	TEQ_{FULL}	0.64
	4	TEQ_{QUAN}	0.64
	4	ln TEQ_{FULL}	0.97
	4	ln TEQ_{QUAN}	0.98
On-post, adults unknowns = adult	7	TEQ_{FULL}	0.14
	7	TEQ_{QUAN}	0.14
	7	ln TEQ_{FULL}	1.00
	7	ln TEQ_{QUAN}	1.00

5.2.2.2 *Statistical Analysis*

Student's t-tests were conducted on the owl data using either untransformed data for juveniles or ln-transformed data for adults (**Table 16**). There was not a statistically significant difference ($p < 0.05$) in TEQs between on-post and off-post reference liver samples for juvenile owls. For the adult owls, differences in TEQs were statistically significant ($p < 0.05$) when the unknown aged owls were excluded, but were not significant when the unknown aged owls were included as adults. These statistical tests were only conducted on the more conservative TEQ_{FULL} datasets, as explained in the decision criteria (**Appendix** B), since the TEQ_{QUAN} dataset was intended to be used for quantitative congener profile analyses—if possible.

5.2.2.3 *Power Analysis*

To determine whether the experimental design had sufficient power to detect the desired differences a power analysis was carried out (Table 17). This analysis was used to determine

whether the observed lack of significant difference (seen in two of the three comparisons shown in Table 16) was due to a limited sample size or greater variance or a smaller minimal detectable difference to be tested, rather than to the actual lack of a significant difference between the mean TEQs of compared groups. None of the possible sample comparisons had sufficient statistical power to conclude that there was a lack of significant differences based upon available data.

Table 16. Statistical significance (*p*) of Student's t-tests for differences in means of TEQ concentrations (ppt) for great horned owl liver data
(values are for separate variance estimates, with values for pooled variances provided in brackets)

Age Class		Number (O, R)	*p*			
			TEQ_{FULL}	TEQ_{QUAN}	ln TEQ_{FULL}	ln TEQ_{QUAN}
Adults	unknowns excluded	4,5	NA	NA	0.03 (0.03)	0.02 (0.03)
	unknowns as adults	7,5	NA	NA	0.08 (0.08)	0.07 (0.06)
Juveniles		9,5	0.43 (0.53)	0.18 (0.27)	NA	NA

A test of power analysis for a minimum of a 15 ppt difference was not conducted because the means (O compared to R) were already different by at least 15 ppt in all cases; hence, there was no point in conducting a power analysis for off-post reference values plus 15 ppt. It is considered more likely, and usually the case, that the small sample size contributed most to the lack of power per the BAS requirements. Had either the minimal detectable difference or group sample sizes been greater than 15 ppt or four to nine individuals, respectively, or had the variances (or standard deviations) been smaller, then more power would have been attained.

Table 17. Power analysis for statistical differences (*p*) between TEQ concentrations (ppt) in different age classes of great horned owls collected from on-post (O) and off-post reference (R) locations
(means and standard deviations were used with α = 0.05 to determine β and power; U = unknown)

Age Class	Number O, R	Mean O (ppt)	Mean R (ppt)	Standard Deviation O	Standard Deviation R	Alpha (α)	Beta (β)	Power (1-β)
TEQ_{FULL}								
Adult	4, 5	157.2	36.0	115.7	48.2	0.05	0.38	0.63
U = Adult	7, 5	110.1	36.0	103.7	48.2	0.05	0.46	0.54
Juvenile	9, 5	31.5	22.1	30.9	11.6	0.05	0.79	0.21
TEQ_{QUAN}								
Adult	4, 5	156.2	31.4	115.3	44.7	0.05	0.34	0.66
U = Adult	7, 5	110.1	31.4	103.7	44.7	0.05	0.41	0.60
Juvenile	9, 5	29.9	13.0	30.9	12.1	0.05	0.55	0.45

5.2.3 TCDD-EQ Results

The TCDD-EQ concentrations are presented in **Table 18**. As discussed previously in Section 4.3.2, the concentrations of TCDD-EQ in livers from the three emaciated owls that were collected on-post were also adjusted downward by a factor of 8. The data sets used for statistical analysis of TCDD-EQ were the full data set with adjusted data that contained ½ the MDL for values less than the MDL. The quantitative data set could not be used in these analyses due to sample numbers of one or zero in three of the four age/location data groups after elimination of all data below the MDL.

Table 18. Summary of $TCDD\text{-}EQ_{FULL}$ concentrations (ppt) measured in great horned owl livers from on-post (O) and off-post reference (R) locations

Age Class	Location	Number	Mean	Minimum	Maximum	Median
Adult	O	4	489.5	188.0	1,070.0	350.0
Adult (adjusted)	O	4	102.0	32.1	188.0	94.6
Juvenile	O	9	15.5	0.5	119.0	2.5
Unknown	O	3	2.8	0.5	5.0	3.0
Adult	R	5	40.0	0.5	187.0	2.5
Juvenile	R	5	2.8	0.5	9.5	1.0

Note: TCDD-EQ concentrations in emaciated owls were adjusted as described in Section 4.3.2.2.

5.2.3.1 *Distribution Analysis*

As with the TEQ assessment, statistically significant ($p < 0.05$) differences in TCDD-EQ concentrations were detected between adult and juvenile birds. Therefore, age classes were analyzed separately. As with the TEQ results, the distributions of the measured owl TCDD-EQ concentrations, determined by the H4IIE bioassay, were log normally distributed (**Table 19**).

Table 19. Kolmogorov-Smirnov tests for statistical significance (*p*) of deviation from normality for great horned owl liver TCDD-EQ data

(A = adult, U = unknown, J = juvenile, R = off-post reference, and O = on-post)

Measure	Age	Site	*p*
$TCDD\text{-}EQ_{FULL}$	A	R	0.003
	A	O	0.86
	U	O	1.00
	J	R	0.02
	J	O	0.00
$ln\ TCDD\text{-}EQ_{FULL}$	A	R	0.88
	A	O	1.00
	U	O	0.57
	J	R	0.45
	J	O	0.67

5.2.3.2 *Statistical Analysis*

Because of the small sample sizes available, statistical analysis was carried out using both the non-parametric Mann-Whitney U-test (**Table 20**) and the parametric Student's t-test, with only a 1-tail criteria applied to examine if on-post results exceed off-post reference results (**Table 21**). As found with the TEQ analysis, no significant differences were noted between juvenile on-post owl livers and juvenile off-post reference owl livers. There were also only significant differences in concentrations of TCDD-EQ between adult on-post owl livers and adult off-post, reference owl livers when separate variances were used and when owls of unknown age were disregarded.

Table 20. Statistical significance (*p*) of the difference of means by Mann-Whitney U-tests between concentrations (ppt) of TCDD-EQ (bioassay) for on-post (O) and off-post reference (R) populations of great horned owls

(Tests conducted by excluding unknown age owls or by including these owls as adults)

Age	Number (O, R)	*p*
Unknown excluded		
Adult	4, 5	0.09
Juvenile	9, 5	0.46
Unknown as adults		
Adult	7, 5	0.33
Juvenile	9, 5	0.46

5.2.3.3 *Power Analysis*

As with the TEQ assessment, a power analysis was performed on TCDD-EQ results to determine whether the tests had sufficient statistical power (**Table 22**). Only when the unknown age owls are included as adults is there sufficient power to detect a difference between the means.

Table 21. Statistical significance (*p*) of Student's t-test for differences in mean concentrations (ppt) of TCDD-EQ for great horned owl livers

(Values are for separate variance estimates with pooled variances provided in brackets)

Age	Number (O, R)	*p*
Unknown excluded		
Adult	4, 5	0.05 (0.05)
Juvenile	9, 5	0.45 (0.50)
Unknown as adults		
Adult	7, 5	0.40 (0.39)
Juvenile	9, 5	0.45 (0.50)

5.2.4 Pattern Analysis

The results of the PCA for great horned owl liver samples are presented in **Figure 11**. No distinct cluster was apparent; in fact, the samples ordinate in different directions away from the central cluster. Without multiple samples representing each distinctive congener profile, it was difficult to identify a common source or profile of congeners. The lack of a consistent pattern of contamination indicates that the profile analysis would not be appropriate. Profile analysis is used as a statistical analysis of the quantitative difference between two groups of samples that share two distinct contaminant profiles. Because there was no unified pattern to distinguish the outlying samples in this analysis, follow-on profile analysis could not be conducted.

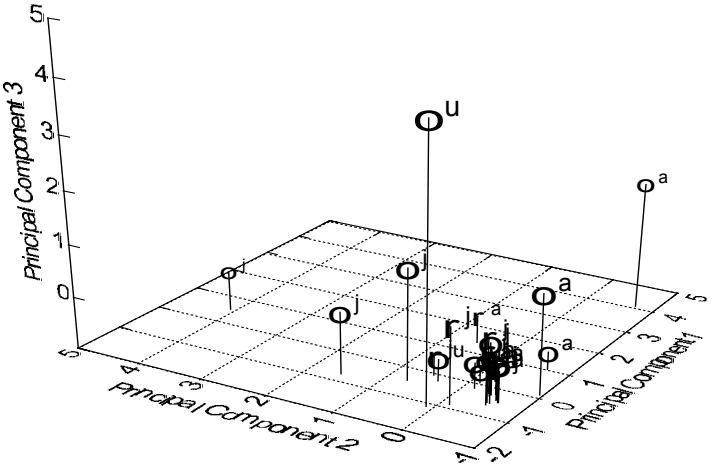
Table 22. Power Analysis for differences in TCDD-EQ concentrations (ppt) in great horned owl livers

(O = on-post, R = off-post, U = unknown)

Measure	Number (O,R)	Mean O (ppt)	Mean R (ppt)	Standard Deviation O	Standard Deviation R	Alpha (α)	Beta (β)	Power (1-β)
$TCDD\text{-}EQ_{FULL}$								
Adult	4, 5	102.3	40.0	71.8	82.2	0.05	0.67	0.33
U = adult	7, 5	59.7	40.0	73.5	82.2	0.05	0.89	0.11
Juvenile	9, 5	15.5	2.8	38.9	3.8	0.05	0.75	0.25
15 ppt Increase $TCDD\text{-}EQ_{FULL}$								
Adult	4, 5	117.3	40.0	71.8	82.2	0.05	0.56	0.44
U = adult	7, 5	74.7	40.0	73.5	82.2	0.05	0.81	0.19
Juvenile	9, 5	30.5	2.8	38.9	3.8	0.05	0.32	0.68

Figure 11. PCA results of PCDD/F profiles in great horned owl livers

(o^u = on-post, unknown age, o^a = on-post adult, o^j = on-post juvenile, r^a = off-post reference adult, and r^j = off-post reference juvenile)



5.2.5 Conclusions for Owls

The outcome is ***Inconclusive*** for the owl liver data. The owl data do not fully meet the data acceptability criteria as set forth in the Decision Procedure. Uncertainty with this data set is due to confounders such as small sample size, deficits in spatial representation, and uncertain residency status for adult owls that can range off-post. However, even though the data have a high level of uncertainty, the data were determined to be useable and sufficient for semi-quantitative analyses. The analysis and interpretation of the owl liver data was made difficult by the many uncertainties introduced because of the use of fortuitous samples and because of the limited number and non-representative spatial distribution of the samples. While many of the uncertainties, such as the patchy spatial distribution of the samples, could not be addressed in the given Tier I Field Screening Study, professional scientific judgment was used to minimize the uncertainties associated with emaciation and unknown owl ages.

To address the effect of emaciation on the concentrations of TEQ and TCDD-EQ in liver tissue, a literature search and whole-body analysis of the available owl carcasses were conducted. Based on these efforts and scientific judgment, a reduction of 8-times the measured concentration was used to downward adjust the PCDD/F concentrations in liver tissues from the emaciated owls.

In addition, the presence of owls of unknown age became a noteworthy issue when it was determined that there was a statistically significant difference in the liver concentrations depending upon the age class of owls. Thus, to address this uncertainty the unknown-aged owls were treated as adults in one set of analyses and excluded from the adult group in a second set of analyses.

Excluding the unknown-aged owls from the statistical analysis resulted in barely rejecting the null hypothesis ($p = 0.05$), i.e., the on-post adult owl liver concentrations are greater than the off-post reference owl liver concentrations. If the unknown-aged owls are included as adults, the difference in TEQ liver concentrations between the on-post and off-post adult owls was no longer significant at the designated level ($p < 0.05$), but with a p value of 0.06, the results indicate that the groups are close to being significantly different.

Because the Decision Procedure could not anticipate all the possible outcomes and thus did not provide guidance on the interpretation of the results, scientific judgement was used to determine the outcome of TEQ analysis for owl livers. The decision for the chemical analysis in owls was that concentrations of TEQs in livers of adult on-post owls (excluding unknown age owls) were greater than concentrations in off-post reference owl livers; i.e., reject the null hypothesis (H_o) (see Step II in text box below). This decision was made even though two of the three comparisons were not statistically significant as shown in **Table 16**.

In addition, the TCDD-EQ analysis resulted in an ***Inconclusive*** outcome because the statistical power was not adequate to see a difference in the on-post versus off-post reference samples even when the unknown-aged owls were assumed to be adults. The statistical analysis also had insufficient power to detect the pre-determined difference of 15 ppt in any of the tests. Analysis of the results could determine how great a difference beyond 15 ppt that the samples could have detected with a power of 80%, but this was not done nor deemed essential by the BAS scientists

for the purposes of this Tier I Field Screening Study (this could be done if the Tier I Field Study is pursued).

Finally, there was no evidence from the PCA that a specific PCDD/F congener profile was present in on-post samples compared to the off-post reference samples. Thus, the outcome of the pattern analysis was to accept the null hypothesis (H_o).

Step I: Data Acceptability	Step II: TEQ ($H1_o$ or $H2_o$)	Step III: TCDD-EQ ($H3_o$ or $H4_o$)	Step IV: Pattern Analyses ($H5_o$)	Step V: BAS's Answer for Overall Decision
No	Reject H_o	Inconclusive	Accept H_o	YES or Inconclusive

5.3 Carp Eggs

Carp are a suitable representative aquatic species on the RMA. Carp eggs were chosen for analysis because carp are bottom feeders and were expected to bioaccumulate persistent organic contaminants such as PCDD/Fs as they have been shown to do effectively for OCPs at the RMA.

5.3.1 Samples Collected

Carp eggs were collected from 16 sexually mature females taken from Lower Derby Lake and from two carp at an off-post reference location.

5.3.2 TEQ Results

Concentrations of TEQ derived from PCDDs and PCDFs in carp eggs were low with mean concentrations for off-post reference samples and on-post samples of 0.6 and 0.9 ppt (**Table 23**). These values compare favorably with the greatest TEQ concentration detected in off-post reference samples of eel tissue that were collected from a relatively pristine New Zealand environment (maximum 0.4 ppt TEQ) (Buckland et al. 1998).

Table 23. Summary of TEQ concentrations (ppt) in carp egg samples

Measure	Group	Number	Mean	Minimum	Maximum	Median
TEQ_{FULL}	O	16	0.9	0.6	1.1	0.9
TEQ_{QUAN}	O	10	0.2	0.1	0.5	0.2
TEQ_{FULL}	R	2	0.6	0.5	0.6	0.6
TEQ_{QUAN}	R	2	0.2	0.1	0.3	0.2

5.3.3 TCDD-EQ Results

Mean concentrations of bioassay-derived TCDD-EQs in carp eggs from the RMA ranged from 6.3 to 22.7 ppt TCDD-EQ, while the mean concentrations in carp eggs from off-post reference areas ranged from 0 to 1.3 ppt TCDD-EQ for the full data set (**Table 24**).

5.3.4 Pattern Analysis

Because of the small number of off-post reference samples and the small concentrations of PCDDs and PCDFs measured in the carp eggs, pattern recognition techniques could not be validly applied to the carp data set.

Table 24. Summary of TCDD-EQ concentrations (ppt) in carp eggs from on-post (O) and off-post reference (R) locations

Measure	Location	Number	Mean	Minimum	Maximum	Median
$TCDD\text{-}EQ_{FULL}$	O	16	6.3	0.5	38	2.5
$TCDD\text{-}EQ_{QUAN}$	O	3	22.7	2	38	28
$TCDD\text{-}EQ_{FULL}$	R	2	1.3	1	1.5	1.3
$TCDD\text{-}EQ_{QUAN}$	R	0	NA	NA	NA	NA

5.3.5 Conclusions for Carp Eggs

The decision matrix resulted in an outcome of ***Inconclusive***. The limited data set for these samples, and the high frequency of MDL values, particularly from off-post reference locations, precluded the meaningful use of statistical tests and pattern analysis techniques. Concentrations of TEQ and TCDD-EQ were sufficiently small that further analysis would not be warranted.

Step I: Data Acceptability	Step II: TEQ ($H1_o$ or $H2_o$)	Step III: TCDD-EQ ($H3_o$ or $H4_o$)	Step IV: Pattern Analyses ($H5_o$)	Step V: BAS's Answer for Overall Decision
No	Inconclusive	Inconclusive	not applicable	Inconclusive

6.0 DISCUSSION

In this section, all the decision steps were combined to yield a final decision. The structure of the Decision Procedure is presented in tabular and graphic forms in Section 3.0, and a synopsis of the procedure is included as **Appendix** B. The Decision Procedure was pre-designed to allow for proper, and hopefully less equivocal, integration of study results from different methods of analysis (TEQs, TCDD-EQs, and PCA/pattern analysis) and from the different species analyzed. The principal question was

Are concentrations of PCDD/Fs in representative biota samples collected on RMA greater than those in comparable samples from off-post reference sites?

It should be noted that the following section requires perspective on the scientific caveats related to the design and results of this Tier I Field Study; please refer to footnotes for the decision matrix found in the summary tables in Section 3 and detailed in **Appendix** B. Briefly, these main caveats are listed here as follows:

- "Concentration" in this context means toxic-equivalents of TCDD generated by the 17 PCDD/F congeners that have Ah-R agonist activity. It is important to note that only Step II (TEQ) provided a direct measure of PCDD/F concentration (and indirect activity); however, Step III (TCDD-EQ) provided an indirect measure (with direct activity).
- In this study, an inconclusive decision indicated that the general question posed could not be answered "yes" or "no" with confidence. An inconclusive outcome would likely result in further analysis by the BAS. In this study, small sample numbers generally caused or were believed to contribute most to the inconclusive results. Therefore, further assessment by the BAS with the available data would be difficult.
- While TCDD-EQ results alone cannot answer the general question posed in the Tier I Field Study, TCDD-EQs could be used in a weight-of-evidence approach to help guide both the interpretation of toxicological significance (especially if PCDD/Fs are the predominant cause of Ah-R activity) and possible future studies at the RMA. The BAS generally recognized that TCDD-EQs, if not overshadowed by other Ah-R activity, could potentially show differences (similar to TEQs) in exposures to PCDD/F concentrations between on-post samples and off-post samples.

6.1 Results for American Kestrel Eggs and Great Horned Owl Livers

For American kestrels, the null hypothesis was not rejected in either Step II or III, nor did the pattern analysis distinguish unique sources on-post compared to off-post reference areas. In addition, independent tests that compared concentrations in the core and peripheral areas, relative to the concentrations at off-post reference locations were conducted. There were no significant differences in concentrations between samples of kestrel eggs that were collected from the core and the peripheral areas of the RMA. The PCA results did not indicate any consistent pattern of congeners associated with the core or periphery of the RMA that would distinguish these samples from samples collected in off-post reference areas. Therefore, data for the American kestrel resulted in the answer,

No, concentrations of PCDD/Fs in American kestrel egg samples collected on the RMA are not greater than those in comparable samples from off-post reference sites

The data for great horned owls were considerably more complex than for American kestrels and a substantial amount of scientific judgment had to be applied to the interpretation of results for the owls, due mainly to complications caused by emaciation and disparate ages of the fortuitous specimens. Despite the comprehensive nature of the Decision Procedure, situations were encountered in the assessment of the owl data that were not considered in the procedure. Treatments of these situations were conducted based on the best scientific judgement with a tendency to be conservative; i.e., where uncertainty existed, decisions about science issues were made to ensure protection.

As has already been mentioned, three of the four adult owls collected on the RMA showed signs of advanced emaciation, which appeared very likely to have resulted in a major redistribution of PCDD/Fs in the body from typical fat stores to the liver, resulting in anomalously elevated concentrations of these chemicals in the liver. These elevated concentrations are likely false positive elevations due to disease. The Decision Procedure also did not account for dealing with the occurrence of owls of unknown ages.

In addition, there was a statistically significant difference in TEQs between adult (adjusted for emaciation) and juvenile owls on-post, again possibly because of the effects of emaciation in the adults. The difference in the age classes meant that statistical analyses had to be carried out separately on the two age classes. This division by age further reduced sample sizes and consequently reduced the power of the statistical analyses. There was also no indication in the Decision Procedure of how to interpret different decision outcomes for adults and juveniles owls.

After consideration of all the available evidence it is the opinion of the BAS that

It is inconclusive whether concentrations of PCDD/Fs in great horned owl liver samples collected on RMA are greater than those in comparable samples from off-post reference sites.

However, if there were any differences that were unable to be statistically detected with the available data, then based on the observations of TEQs in terms of adjusted magnitude and spatial scales, these differences would not be expected to be great, thereby minimizing the likelihood of both exposure for biomonitoring of sources and any associated toxic risks on ecological scales. Furthermore, the thorough evaluation of TCDD-like PCBs did not indicate that these congeners were risk drivers in most cases, nor were they necessarily required to resolve mass balance differences between results for TEQs and TCDD-EQs; however, this potentially valuable need to account for PCB contributions to TEQs and risks could not be ruled out ahead of time.

The next step of the Decision Procedure for the terrestrial ecosystem was to combine the results for the owls and kestrels. The Decision Procedure stated

> If the overall outcome for either or both species is inconclusive, without a "yes" for either, the conclusion of the Tier I Field Study is ***Inconclusive*** for the terrestrial environment.

American Kestrel Decision	Great Horned Owl Decision	Overall Terrestrial Species Decision
NO	Inconclusive	Inconclusive

Therefore, the conclusion for the terrestrial environment was *Inconclusive*.

Although the presence of three adult owls with elevated concentrations of PCDD/Fs in the liver would indicate that PCDD and PCDFs may be elevated on the RMA, the level of uncertainty in this finding was high. In addition, the remaining findings from this study indicated that PCDD/Fs should not be considered as COCs at the RMA.

The BAS also considered other studies that could provide information on exposures to PCDD/F at the RMA. These studies included

1) *Induction of Immunotoxicity and Mixed-Function Oxygenase Activity as Biomarkers of Exposure to Environmental Contaminants in the Deer Mouse* (*Peromyscus maniculatus*) (Gard 1995).

2) *Characterization of Dioxins, Furans and PCBs in Soil Samples Collected from the Denver Front Range Area* (EPA 2000a).

3) *Characterization of Dioxins, Furans and PCBs in Random Soil Samples Collected from the Rocky Mountain Arsenal* (EPA 2000b).

4) *Characterization of Dioxins, Furans and PCBs in Soil Samples Collected from Historic Use Areas of the Rocky Mountain Arsenal* (EPA 2000c).

5) *Results of a Survey of Fortuitous Specimens and Soil Samples for Rocky Mountain Arsenal for Trace Organic Contaminants, Arsenic, and Mercury* (EcoLogic 1996).

The Gard study looked for possible specific biomarkers of PCDD/F exposure in deer mice at the RMA. Little indication of PCDD/F exposure was found under the study conditions.

The Denver Front Range dioxin soil study collected soil samples that were associated with locations near certain wildlife collection locations on the RMA and at several off-post reference areas. The highest off-post owl and kestrels were associated with low (approximately 1- to 2-ppt TEQ) background soils. Thus, no association between PCDD/F concentrations in soil and biota tissues could be determined from this outcome in off-post reference samples. There were small elevations of PCDD/F in soils at the RMA in areas that were co-located with elevated owl liver concentrations, but the data did not permit robust correlation analysis. The soil study results did not contradict findings of the Tier I Field Study in biota, and were generally consistent with the conclusion that a possible small source of PCDD/Fs existed on the RMA with low concentrations.

The results of the current study were in general agreement with those of the previous CDPHE study (EcoLogic 1996). In both studies, the concentration of PCDD/Fs was increased in the livers of emaciated great horned owls. While these observations indicated that exposure of owls in the current study did not provide evidence of more widespread contamination of the terrestrial ecosystem, it also did not provide conclusive evidence of a RMA origin of the PCDD/Fs present in owl tissues. The absence of significant concentrations of PCDD/Fs in juvenile owls and kestrels was in agreement with earlier observations from red-tailed hawk and small mammals that demonstrated no significant accumulation of PCDD/F in the terrestrial ecosystem of the RMA.

6.2 Results for Carp

The decisions for both TEQ and TCDD-EQ for carp were ***Inconclusive***. The small concentrations of PCDD/Fs should probably have resulted in a "no" finding (i.e., PCDD/Fs are not COCs at the RMA), but the lack of statistical power as designated in the Decision Procedure meant that an inconclusive finding must be made. While the statistical power of the analyses was lower than required, the concentrations of PCDDs and PCDFs measured in the fish from on-post were as small as would be expected for background concentrations of these compounds in unimpacted locations. Therefore, the best scientific judgement of the BAS was that these chemicals were not a cause for concern in the aquatic environments of the RMA.

7.0 CONCLUSIONS

The current study examined the possibility of a bioavailable source of PCDD/Fs on the RMA. The conclusion that PCDD/Fs are not COCs was based on results from analysis of PCDD/Fs (both chemical-specific and bioassay methods), pattern analysis, and toxicity considerations.

Multiple lines of evidence were used to reach the final decision. Concentrations of PCDD/Fs were present at background concentrations in carp collected from the aquatic environment on-post. Similarly, PCDD/F concentrations in American kestrel eggs collected on-post were as small or smaller than concentrations measured in eggs from off-post reference locations in the Denver metropolitan area. Finally, PCDD/F concentrations measured in the livers of juvenile great horned owls collected on-post were not different than concentrations measured in juvenile owls collected from off-post reference locations. However, the three highest concentrations were in the three juveniles collected in the South Plants area of the RMA. Only adult great horned owl livers contained concentrations of PCDD/Fs that were greater on-post than off-post.

Finally, the pattern of PCDD/F congeners present in the samples from the RMA was compared using principal components analysis to determine whether a common pattern of contaminants on-post could indicate the presence of an on-post source of contamination. No consistent patterns were detected in the samples.

Concentrations of PCDD/Fs were also compared to concentrations that were expected to result in adverse effects in wildlife species (**Appendix** G). Concentrations of PCDD/Fs measured in carp eggs were less than concentrations that would be expected to cause adverse effects either directly in the carp themselves, or in birds feeding on the carp, such as the bald eagle that is known to be particularly sensitive to the adverse effects of these compounds. The average concentrations detected in this study were less than 1 ppt of TEQ compared to predicted effects concentrations of 170 to 1,200 pg/g (wet weight [ww]) for reproductive effects in carp eggs.

Concentrations of TEQ in American kestrel eggs averaged less than 20 ppt at off-post reference locations and less than 10 ppt on the RMA. These values can be compared with a predicted-effects concentration based on a no observed adverse effect level (NOAEL) calculation of 70 pg/g (ww). It therefore seems unlikely that current PCDD/F concentrations in American kestrel eggs on the RMA are causing adverse effects on reproduction.

The toxicity reference value derived for great horned owl liver is 15 pg/g (ww) and is based on several conservative assumptions. Only the average concentration for juvenile owls from off-post reference locations was less than this value. The average concentration of TEQs in adult great horned owl livers on the RMA was approximately 10-fold greater than this NOAEL value, suggesting possible cause for concern. These findings are in general agreement with analyses for other high trophic level organisms on a global scale, which suggested that concentrations of PCDD/Fs in wildlife were relatively close to concentrations known to cause adverse effects (Jones et al. 1996). However, the average concentration of TEQs in livers of adult owls from off-post reference locations was also approximately 2-times greater than this concentration and the average concentration for the three unknown-age owls on the RMA was similar to that observed in off-post reference birds. Taken together, this suggests a possibility of low excess risk to owls on the RMA, as well as potentially in the general Denver metropolitan area.

However, much of this estimated risk is driven by large uncertainty factors due to extrapolations from weak data sets and therefore may not actually reflect adverse effects.

During this Tier I Field Study, corresponding studies of PCDD/Fs and PCBs in soils collected on the RMA and from off-post areas were conducted. Results of these soil studies showed similar findings of small elevations of PCDD/Fs in localized areas of South Plants (Figure 12 [next page] and Map 2 in Section 10). Average concentrations in soils were similar in on-post samples and off-post samples, and all samples were much less than levels of health risk concern.

While the BAS was confident that there was not a large, available source of PCDD/Fs on the RMA, the presence of a small, localized source deserved consideration. The two adult and several juvenile great horned owls with the most elevated PCDD/F concentrations were collected near South Plants, an area that is currently undergoing remediation. This same area also produced a cluster of soil samples with somewhat elevated PCDD/F concentrations (Figure 12, EPA 2000a), further suggesting the existence of a localized source. However, the PCA for owl data did not show any distinct patterns, using adjusted data. While visual inspection of the predominant congeners in the co-located soils and owl tissues at South Plants does suggest some common and perhaps unique patterns, it is an uncertain association; furthermore, soil congener patterns have not yet been evaluated for the soils at the RMA South Plants area. It is probable that any past source of PCDD/Fs in the core area on RMA is currently planned to be remediated along with the other organochlorine pesticide contamination in soils. It was the opinion of the BAS that biomonitoring programs by the USFWS were already in existence that could be used to verify that any potential source areas would be adequately removed, as extrapolated from measured dieldrin concentrations in biota. (Note: Human TEFs were used with the assumption of 100% bioavailability for producing the soil TEQs while bird TEFs were used to produce the owl TEQs. This distinction and assumption for human TEFs also applies to the rest of the soil TEQs at RMA that are shown in Map 2 in Section 10.)

Figure 12 is a map of the central South Plants area of the RMA (see next page) showing in detail the confined low levels of elevated TEQs that were co-located in owl tissues (circles and triangles) and in surface soils (squares).

SP-1
4 ppt

SP-2
21 ppt

SP-3
30 ppt

SP-4
6 ppt

SP-5
2 ppt

SP-6
11 ppt

SP-7
96 ppt

SP-8
32 ppt

SP-9
3 ppt

SP-10
3 ppt

SP-11
2 ppt

SP-12
3 ppt

LEGEND

Relative Ranking **JUVENILE OWL SAMPLE LOCATION - Sampled 1996**

- △ TOXICITY EQUIVALENT (TEQ) < 15
- ▲ TEQ >= 15 and < 40
- ▲ TEQ >= 40 and < 100
- ▲ TEQ >= 100

ADULT OWL LOCATION = EcoLogic, 1996 Study

- ⬠ TEQ < 15
- ⬟ TEQ >= 15 and < 40
- ⬟ TEQ >= 40 and < 100
- ⬟ TEQ >= 100

ADULT OWL LOCATION* - Sampled 1996

- ○ TEQ < 15
- ● TEQ >= 15 and < 40
- ● TEQ >= 40 and < 100
- ● TEQ >= 100

* Adult owl locations include samples from known adults and older birds of unknown age.

KESTREL BOX LOCATION - Sampled 1996

- □ TEQ < 15
- ■ TEQ >= 15 and < 40
- ■ TEQ >= 40 and < 100
- ■ TEQ >= 100
- ☒ Indicates nest box in core area

- ✚ Dioxin Grab Soil Sample Location, Study 2
- ✚ Subsample Location For Composite Dioxin Soil Sample, South Plants Area, Study 4
- ✚ Subsample Location For Composite Dioxin Soil Sample, Historical Source Areas, Study 4

SP-2 2 ppt — Composite Sample Identification Dioxin Concentration TEQ

- Preliminary Approximate Area of Relatively Higher but Generally Small Levels of Dioxin In Owl Tissues
- Human Health and Biota Risk Areas
- Remedial Project Sites
- Streams, Canals, Ditches
- Roads
- Fences
- Buildings
- Lakes and Ponds
- Rocky Mountain Arsenal CERCLA Site

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N

1000 0 1000 2000

Feet

SOUTH PLANTS AND VICINITY TOXICITY EQUIVALENT VALUES FROM COMPOSITE SOIL SAMPLES (PARTS PER TRILLION)

6-26-01 PC35406 e:\gis\rma\ecorisk4.apr SPlants TEQ 8x11

8.0 TECHNICAL RECOMMENDATIONS

It is the best scientific opinion of the BAS scientists that PCDD/Fs are not a contaminant of concern (COC) at the RMA. Any small, localized source of PCDD/Fs in South Plants would very likely be removed by current remediation activities.

Therefore, the evaluation of PCDD/Fs in some samples of the ongoing Biomonitoring Program (USFWS) is recommended. This will provide additional assurance that the remediation of the post will have adequately reduced any potential sources of PCDD/F contamination.

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10.0 MAPS

RMA Map 1 (See next page)

MAP 1. ***BIOTA RESULTS FOR RMA ON-POST AND OFF-POST REFERENCE WILDLIFE IN TIER I FIELD DIOXIN STUDY, INCLUDING CDPHE (ECOLOGIC) RESULTS FROM PRELIMINARY STUDY IN 1995, AND THE SOIL SAMPLING LOCATIONS AT RMA FOR THE DENVER FRONT RANGE STUDY (16 X 22 INCH GIS MAP)***

Off-Post Adult Owl Samples

Location	TEQ (full)
96RFGH03	122
96RFGH12	24
96RFGH05	12
96RFGH07	12
96RFGH01	10

Off-Post Juvenile Owl Samples

Location	TEQ (full)
96RFGH04	34
96RFGH09	32
96RFGH10	24
96RFGH02	12
96RFGH11	8

Off-Post American Kestrel Samples

Location	TEQ (full)
AKEG02AR	201
AKEG01RC	52
AKEG01AR	31
AKEG01ACP	15
AKEG01BL	9
AKEG04BL	7
AKEG04CC	7
AKEG03BL	6
AKEG05ACP	5
AKEG01YP	5
AKEG10CC	4
AKEG07CC	4
AKEG03AR	4
AKEG05CC	3
AKEG08CC	3
AKEG06CC	2

96FGH002
50 (399)

AKEG19NW 16
AKEG20NW 5
AKEG20NE 25
96FGH162 22
AKEG26NW 11
96FGH231* 14
96FGH232* 32
AKEG27NW 25
AKEG25NW 23
AKEG30NW 22
AKEG29NW 3
AKEG29NE 22
AKEG33NW 8
AKEG34NW 3
AKEG35NW 16
AKEG35NE 8
AKEG31NW 3
96FGHL219 7
AKEG32NW 2
AKEG32NE 12
GHL34SE96 18
AKEG35SE 6
96FGH250* 96
96FGHL217 32
GHL31SE96 (location uncertain) 2
AKEG03NW 14
(AKEG3NW) (7)
AKEG04NW 5
AKEG2NW 29
96FGH007 95 (2380)
96FGH026 41
96FGH027* 58
AKEG6NW 4
96FGH017 74 (594)
AKEG32SW 4
AKEG05NE 10
33361 (location uncertain) 109
96FGH044 98
33319 (location uncertain) 88 (589)
33434 (location uncertain) 26
96FGH144 210
96FGH190 6
AKEG11NW 8
AKEG12NW 1
AKEG8NE 31
AKEG11SW 2
AKEG12SW 8
AKEG8SW 3

LEGEND

Relative Ranking

JUVENILE OWL SAMPLE LOCATION - Sampled 1996

- background: TOXICITY EQUIVALENT (TEQ) < 15
- low: TEQ >= 15 and < 40
- medium: TEQ >= 40 and <100
- high: TEQ >= 100

ADULT OWL SAMPLE LOCATION* Sampled 1996

- background: TEQ < 15
- low: TEQ >= 15 and < 40
- medium: TEQ >= 40 and < 100
- high: TEQ >= 100

* Adult owl locations include samples from know adults and older birds of unknown age.

ADULT OWL LOCATION - EcoLogic Inc, 1996 Study

- background: TEQ <15
- low: TEQ >= 15 and < 40
- medium: TEQ >= 40 and < 100
- high: TEQ >= 100

KESTREL BOX LOCATION - Sampled 1998

- background: TEQ <15
- low: TEQ >= 15 and < 40
- medium: TEQ >= 40 and < 100
- high: TEQ >= 100
- Indicates nest box in core area

Sample name and full TEQ values are posted next to sample location. TEQ values in parentheses have not been adjusted for the four emaciated on-post adult owls.

- STREAMS, CANALS, DITCHES
- ROADS
- FENCES
- BUILDINGS
- LAKES AND PONDS
- ROCKY MOUNTAIN ARSENAL CERCLA SITE
- REMEDIAL PROJECT SITES
- HUMAN HEALTH AND BIOTA RISK AREAS
- WESTERN TIER PARCEL
- PRELIMINARY APPROXIMATE AREA OF RELATIVELY HIGHER BUT GENERALLY SMALL LEVELS OF DIOXIN IN OWL TISSUES
- DIOXIN GRAB SOIL SAMPLE LOCATION, STUDY 2
- SUBSAMPLE LOCATION FOR COMPOSITE DIOXIN SOIL SAMPLE, STUDY 3
- SUBSAMPLE LOCATION FOR COMPOSITE DIOXIN SOIL SAMPLE, SOUTH PLANTS AREA, STUDY 4
- SUBSAMPLE LOCATION FOR COMPOSITE DIOXIN SOIL SAMPLE, HISTORICAL SOURCE AREAS, STUDY 4

27 soil samples at the following Biological Advisory Subcommittee and historical composite sample locations:
a) 12 on-post owl sample locations
b) 5 off-post reference soil samples collected at the following locations:
i) Aurora Reservoir
ii) Barr Lake
iii) Cherry Creek Reservoir (2 samples)
iv) Shell Property, section 14
c) 10 historical source area locations

N

1000 0 1000 2000 Feet

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ROCKY MOUNTAIN ARSENAL SOIL SAMPLING FOR BIOLOGICAL ADVISORY SUBCOMMITTEE TIER 2 STUDY AND DIOXIN TOXICITY EQUIVALENT VALUES FOR OWL AND KESTREL SAMPLES

6-1-01 PC35405 e:\gis\rma\ecorisk4.apr OwlKestFullTEQAdjustC

RMA Map 2 (see next page)

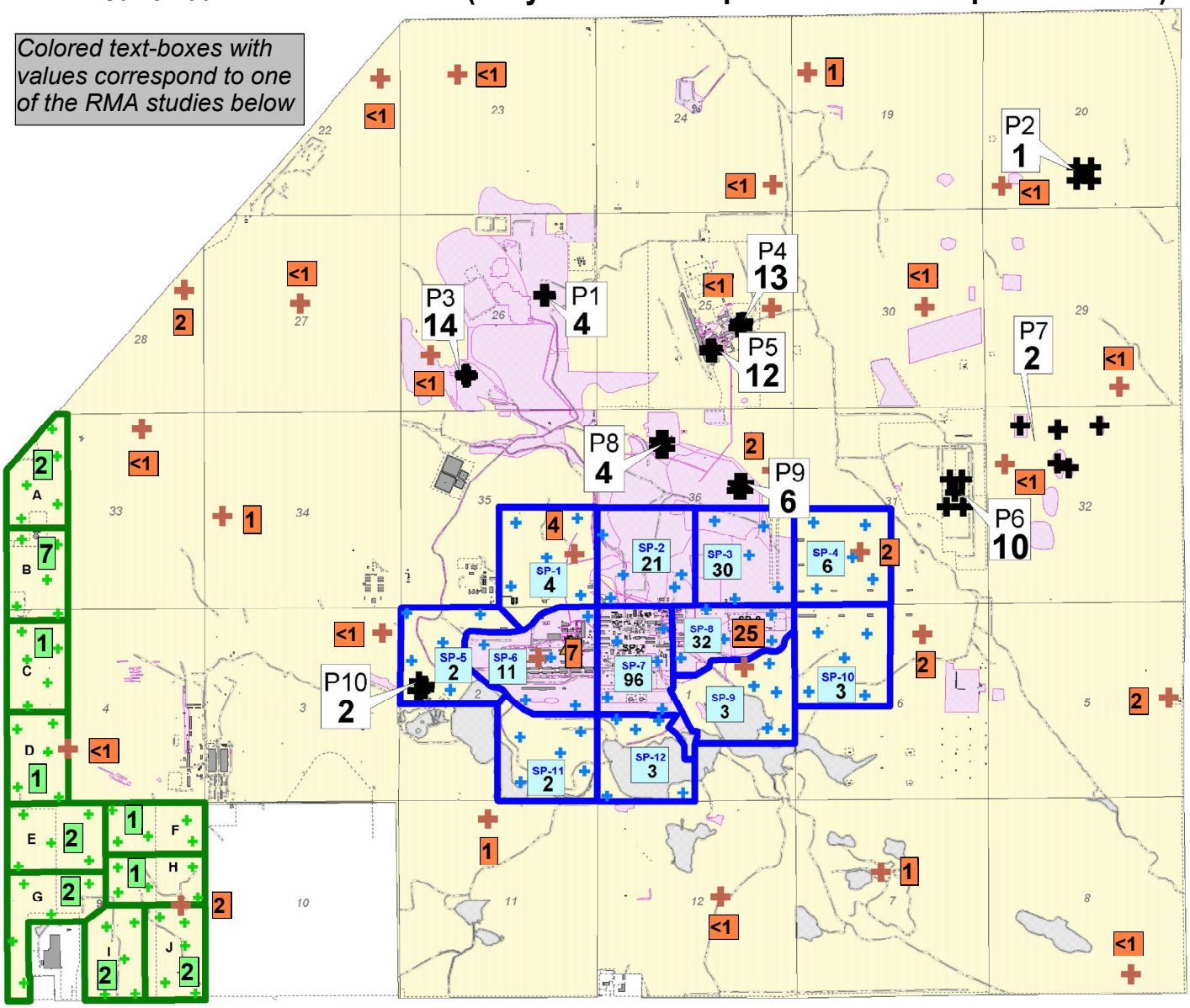
MAP 2: ***TEQ CONCENTRATIONS IN SURFACE SOILS AT RMA FOR 17 PCDD/F CONGENERS***

Results of Dioxin Concentrations (TEQ, ppt) in Soils at the RMA

June 2001

(+ symbols on map denote soil sample locations)

Colored text-boxes with values correspond to one of the RMA studies below



Study 3 - Western Tier Parcel

Study 4a - South Plants Sites

Study 2 - Random Grab Sites

Study 4b - Historic Use Sites

Study 1 - Off-post Random Study (data not shown here)

N

2000 0 2000 4000 Feet

ROCKY MOUNTAIN ARSENAL
CDPHE SAMPLE LOCATIONS
TOXICITY EQUIVALENT VALUES (ppt)
FROM COMPOSITE SOIL SAMPLES

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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10-12-00 L:\gis\rma\dioxrma2.apr StateSoilTEQ

Map was modified to show all soil results at RMA, produced by Gerry Henningsen, USEPA R8